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NUMBER 2

# THE HAWAIIAN PLANTERS' RECORD



Black Widow spider parasite recently introduced into Hawaii from California and distributed throughout the Territory (greatly enlarged).

SECOND QUARTER 1940

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# THE HAWAIIAN PLANTERS' RECORD

Vol. XLIV

SECOND QUARTER 1940

No. 2

*A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the plantations of the Hawaiian Sugar Planters' Association.*

## In This Issue:

### *Parasite of the Black Widow Spider:*

An account is given of the discovery and introduction into Hawaii of a new parasite of the Black Widow spider, including a description of the habits of the parasite and its method of attacking and destroying the eggs of this poisonous spider.

### *Forms of Nitrogen for Sugar Cane:*

Controlled pot tests in which three sources of nitrogen were used on 31-1389 cane have pointed out some interesting interactions with soils and with phosphate fertilization which apparently can modify some of the direct effects of these different nitrogen fertilizers.

### *The Synthesis of Sucrose by Excised Blades of Sugar Cane:*

A leaf cut from a sugar cane plant and placed in a solution of glucose in absolute darkness can continue to make cane sugar for nearly two weeks. Both the amount of glucose absorbed and the amount of cane sugar formed are profoundly affected by temperature.

### *Factors Affecting the Germination of Sugar Cane:*

Results of extensive studies divide factors affecting germination into two general categories: those external to the seed piece, including soil temperature, aeration, and moisture; and those internal to the seed piece, including age and composition, length of seed piece, position of the buds relative to one another, and the presence of trash. Results of experiments dealing with each of these points are presented.

In addition to these studies, many experiments are reported dealing with methods which can be used to stimulate germination. Some simple but very effective methods are available particularly for use under otherwise unfavorable germination conditions.



## Notes on the Life History of *Baeus californicus* Pierce, an Egg Parasite of the Black Widow Spider

By C. E. PEMBERTON AND J. S. ROSA

The Black Widow spider, *Latrodectus mactans* (Fab.), was first noticed in the Hawaiian Islands in 1925. It now occurs in many parts of the Territory and occasionally becomes very abundant in some lowland areas receiving light annual rainfall. Actual records of positive bites by this spider to residents in Hawaii are rare and we have not heard of any serious results from these bites. However, in the average case, the person bitten does not see the spider and is more concerned for the moment in the bite than in the offender and the spider escapes unseen and unrecorded. There have been cases of serious illness lasting for a week or more in Hawaii following bites by something not actually seen and these cases have occurred in localities where the spider is known to be present. The symptoms have resembled those attending actual Black Widow bites. A recent case occurred in which the patient was bitten on the foot with attending symptoms of a Black Widow spider bite, resulting in hospitalization and recurrent illness over a period of about two weeks. There is reason to assume that this spider is just as poisonous in Hawaii as elsewhere. Corroborative evidence in support of this belief was obtained in Honolulu by Dr. Nils P. Larsen, who reported to the Hawaiian Academy of Science on May 16, 1928, the results of his experiments in exposing guinea pigs to attack by this spider.

A closely related species, *Latrodectus geometricus* (L.), identified by Dr. F. X. Williams, was found at Waikiki by W. L. McCleery during 1939. Entomologists later found it rather common on other parts of Oahu. This is also an undesirable immigrant to Hawaii, but it is not considered as dangerous as the Black Widow.

As with insects, spiders are also subject to control by various biological factors, including insect parasites and predators. A few definite natural enemies of the Black Widow spider are known. One, a metallic-blue-and-green wasp, *Chalybion caeruleum* (Linn.), first seen in Honolulu in 1931 by Dr. Williams, is known to capture, anesthetize, and store Black Widow spiders in the empty mud cells made by the black-and-yellow, mud-daubing wasp, *Sceliphron caementarium* Drury. These spiders then serve as food for the developing young of the blue-and-green wasp. It is probable that the *Sceliphron* wasp may also use Black Widow spiders, among others, in the same way. Unfortunately these beneficial wasps are in turn preyed upon by a number of parasitic and predatory foes, which greatly decimate their ranks at times, and thus prevent a thorough and continuous suppression of the spider. Apart from these spider-catching wasps, no important enemies of the Black Widow occurred in Hawaii until the recent introduction of the parasite discussed in the present paper.

During 1938, Dr. W. Dwight Pierce, senior curator of entomology of the Los Angeles museum, together with his associates, Dorothy Pool and Charles A.

Fleschner, discovered a new egg parasite of the Black Widow spider in clumps of cactus on the sand dunes at El Segundo, Los Angeles County, California. In a recent publication (Bulletin of the Southern California Academy of Sciences, Vol. 37, Part 3, September–December, 1938, pp. 101–104) Dr. Pierce has described this parasite and given some interesting facts concerning it. It is quite similar to one previously known in Haiti (*Baeus latrodecti* Dozier). The parasite appeared to be worthy of trial in Hawaii and arrangements were made with Dr. Pierce for its introduction. This was successfully accomplished during August 1939. Two consignments, comprising a total of 15 lots of parasitized eggs, were prepared by Dr. Pierce and turned over to Mr. Swezey for transmittal to Hawaii, Mr. Swezey being in California at that time. Several hundred parasites, representing both sexes, emerged from the eggs after arrival at the H. S. P. A. quarantine room in Honolulu. From these the junior author has succeeded in breeding large numbers of parasites, which have been liberated in various parts of the Territory. Between August 21, 1939, and April 1, 1940, about 32,500 have been distributed.

The female of this new parasite is wingless, as shown in the illustration on the cover. She is, however, quite active and when disturbed may jump 50 or more times her own length. She measures about  $1/33$  of an inch in length. The male is winged, slightly larger, and outnumbered by the females at a ratio of about 10 to 1. The parasite is apparently strictly specific on the eggs of the Black Widow spider. All attempts to breed it on the eggs of *Latrodectus geometricus* have failed. The females have exhibited no interest whatsoever in any eggs excepting those of the Black Widow spider, and over these she shows intense concern when allowed access to them. Her small, compact, wingless body, free of encumbering hairs or long spines, enables her to manipulate with ease amongst the loosely woven threads of web, which constitute the spider's retreat, insect snare, and domicile for her egg sacs and newly hatched young.

The spider's egg sac is a semicircular, silken body about one-half inch or less in diameter enclosing usually from 200 to 400 pinkish-white spherical eggs. Some of these egg sacs and the enclosed eggs are shown in the accompanying figures. The egg sacs can be found suspended in thin threads of web under stones, boards, piles of rubbish, beneath buildings, in tree stumps near the ground, sometimes suspended in clumps of grass or weeds and in various other protected places. By crawling and jumping, the parasite apparently has no difficulty finding and reaching egg sacs in any of these positions.

Upon locating an egg sac the parasite carefully investigates the surface for several hours, as shown in Fig. 1 (upper). In some observed cases in the laboratory she may continue this preliminary investigation for more than 24 hours. If the egg sac is over three days old, she will abandon it without further interest. The egg sac must be somewhat less than this age to arouse her full attention. Finally, if all is to her satisfaction she proceeds to cut her way into the interior of the sac, leaving a neat, circular entrance hole about  $1/60$  of an inch in diameter. Fig. 1 (lower) shows a female boring her way into the sac with half of her body already in.

Immediately upon gaining entrance into a satisfactory egg sac the parasite may begin inserting her own eggs into those of the spider. Dissection shows that she has at birth well over 200 mature eggs packed in her egg tubes. Her prime motive in life is now to insert these individually in separate spider eggs, of which

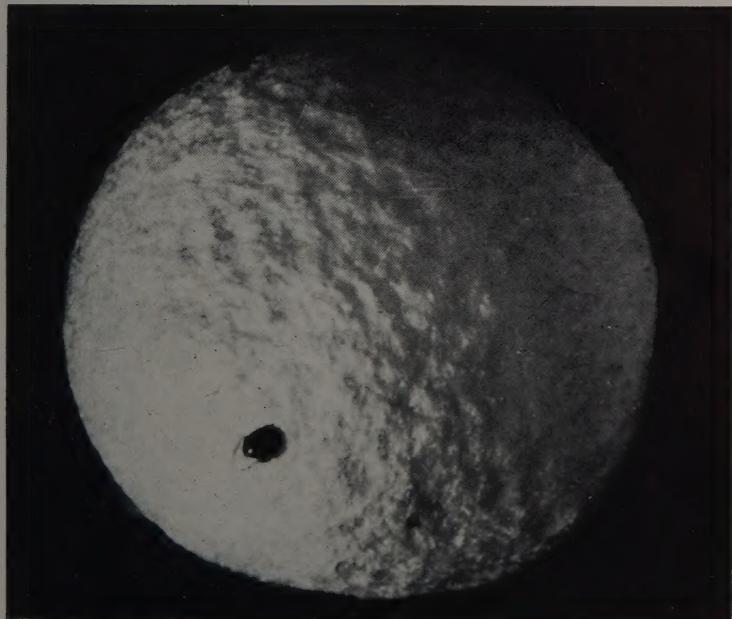


Fig. 1. Upper—Parasite investigating surface of egg sac of Black Widow spider. Lower—Parasite boring into egg sac of Black Widow spider.

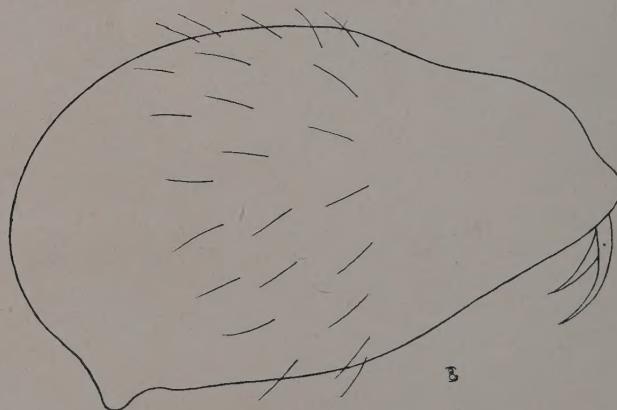
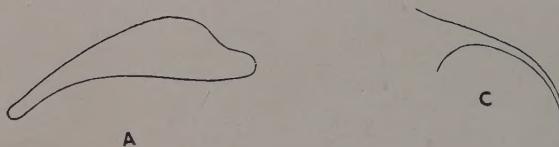
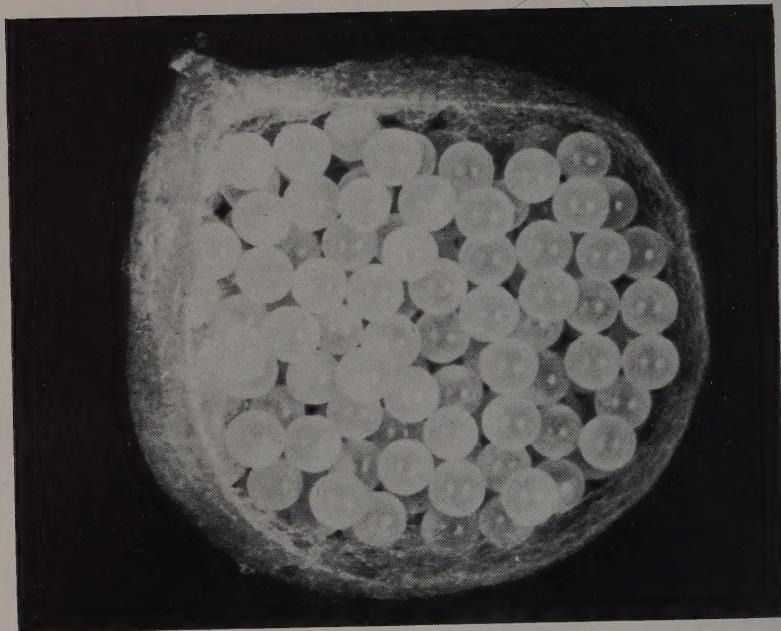


Fig. 2. Upper—Egg sac of Black Widow spider cut open to show contents.  
Lower—*A*. Parasite egg. *B*. Newly hatched parasite larva. *C*. Mandible of newly hatched parasite larva.

there is an abundance in any egg sac she may enter. Fig. 2 (upper) shows a fresh spider egg sac cut open to present the problem confronting her. With her sharp sting or ovipositor she quickly drills a microscopic hole into a spider egg and inserts her own egg. She then immediately withdraws her ovipositor and within a few seconds has moved to another spider egg to repeat the operation. The whole process of laying a single egg usually requires from two to nine minutes. Such observations are made possible by cutting the top off of an egg sac and watching through a microscope the operations of the parasite, who remains oblivious to such invasion of her privacy. Females watched in this manner for a half hour continued laying without pause. Assuming an average of five minutes per oviposition, about 16 hours would be required for the deposition of all of her eggs. Actually more time than this is probably required. Sometimes the exhausted female may emerge from the egg sac a few days after completing her work, but many of them remain in the sac to die among the eggs which they have parasitized.

Dr. Pierce found that this parasite would live from 10 to 14 days, even without food. The writers were able to keep females alive a little longer than this if honey and water were supplied for food and no opportunity was given the parasites to oviposit. At best, few lived 20 days.

The general appearance of the newly laid parasite egg is shown in Fig. 2 A (lower). At that time it measures 0.16 mm. in length, or about  $1/157$  of an inch. This is found floating free in the liquid interior of the fresh spider egg. More than one egg may be deposited in a single spider egg, but only one individual survives to develop to maturity although all may hatch. This happens when more than one parasite enters an egg sac and is a common occurrence in laboratory breeding work at least. Under Honolulu temperatures during January 1940, the egg was found to hatch about three days after deposition.

The newly hatched larva of the parasite is shown in outline in Fig. 2 B (lower). It measures about  $1/63$  of an inch in length and exhibits extreme simplicity in structure. Externally it has the appearance of an ovoid, transparent body, bearing a peripheral band of fine elongate setae and at the anterior end a pair of long, slender, curved mandibles, capable of rather weak movement. Fig. 2 C (lower) shows one of the mandibles removed. The larva lies completely immersed in liquid food within the spider egg. Its main function is now one of food absorption with consequent great degeneracy in body structure. It grows rapidly and within 10 days has swelled sufficiently to occupy the entire interior of the spider egg. Superficially it now resembles a simple globular membrane filled with liquid.

When about 13 days old the larva pupates and 10 days later has matured into a perfect parasite tightly doubled up in the spider egg. This is about 25 days after the parasite egg was laid. By cutting open the spider egg sac a clear view can now be obtained of the next momentous phase in the life of the parasite. Under the microscope a dark reddish parasite can be clearly seen through the thin eggshell of each spider egg. When the final moment for emergence arrives the sharply pointed mandibles of the parasite can be seen rapidly opening and closing against the interior of the egg, together with considerable movement of the parasite head. Within a few minutes the mandibles cut a neat half circle through the

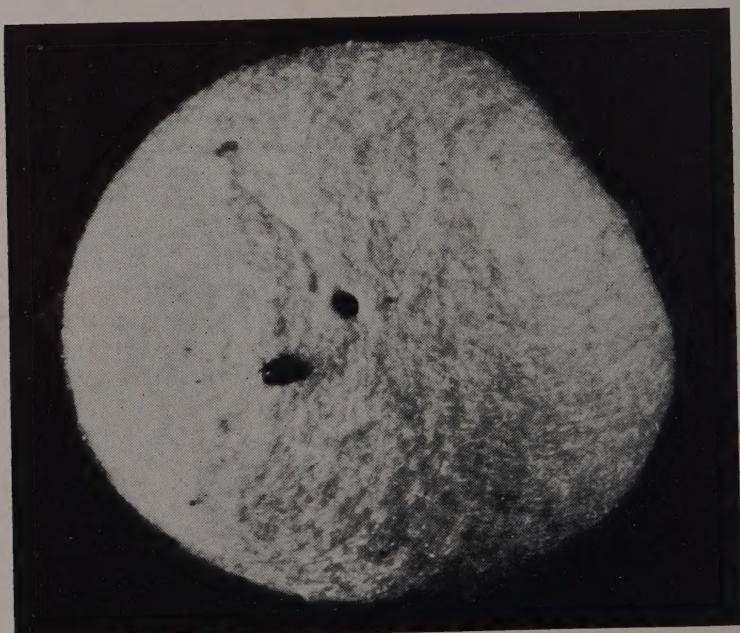
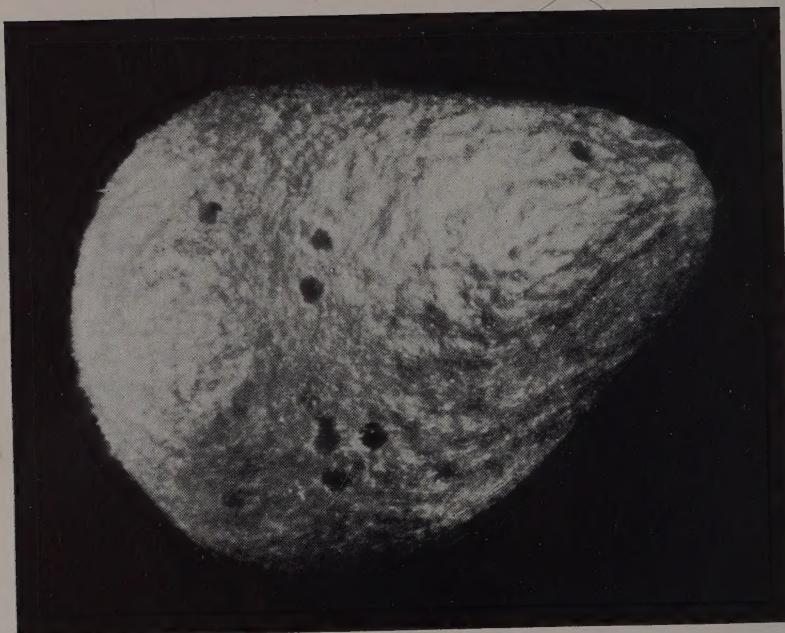


Fig. 3. Upper—General appearance of Black Widow spider egg sac showing emergence holes made by escaping parasites. Lower—Showing parasite emerging from Black Widow spider egg sac.

spider eggshell and the parasite head quickly protrudes followed by the body and the parasite is soon out and moving about inside the spider egg sac.

Within a short while following the hatching of the first parasite, more individuals gnaw and break their way out of the spider eggshells. The interior of the egg sac rather suddenly presents a state of considerable activity. Both sexes emerge at about the same time, though most of the males hatch before all of the females have emerged. Males can be seen pairing with the females almost before the latter are completely extricated from the spider eggshells. Since males are greatly outnumbered by the females, pairing is of but a moment's duration in order that all of the females may become fertilized before finally escaping from the egg sac. Mating does occur to some extent outside the egg sac, but this is the exception rather than the rule. At least several hours after the first parasite has hatched, some of the females start cutting their way out of the egg sac to escape into the outer world. A small number will usually escape the first day, but the mass evacuation generally comes the second day or slightly before that, with stragglers coming out for several days thereafter. Usually an average of about 9 emergence holes are cut, through which the mass of the parasites escape. Almost invariably a number of males die in the sac without attempting to escape. Those that do escape usually use the exit holes made by the females. An evacuated egg sac on which about 9 emergence holes can be seen is shown in Fig. 3 (upper). In Fig. 3 (lower), emergence from the sac can be seen, with a female just coming out. Even at this early period in her short life she is ready to begin parasitizing spider

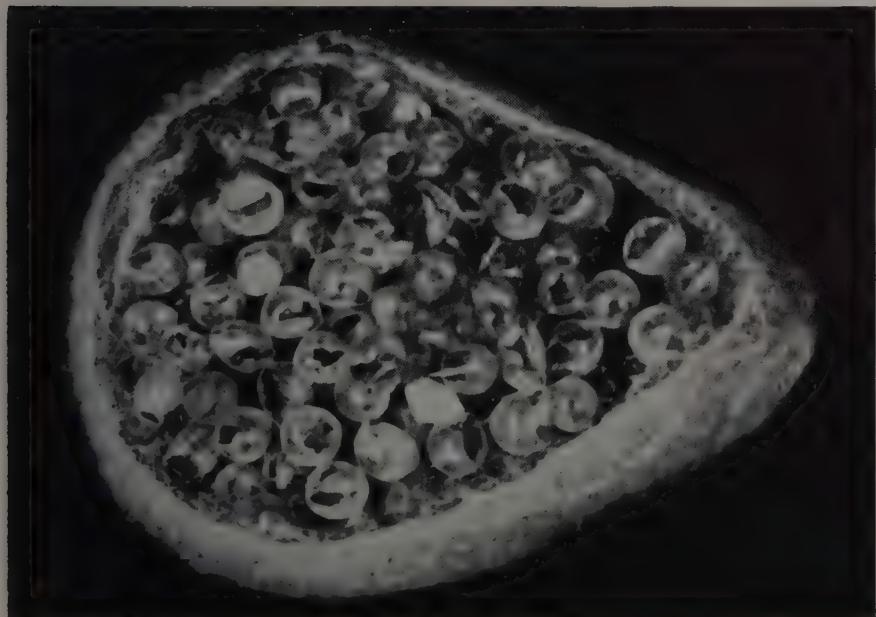


Fig. 4. Interior of egg sac of Black Widow spider after eggs have been parasitized and the parasites have escaped.

eggs. If she is unfertilized all of her progeny will be males; if fertilized, most of the progeny will be females. The appearance of the interior of a parasitized egg sac after all of the parasites have emerged is shown in Fig. 4.

Should any spider eggs in the sac be unparasitized, the young spiders come out of these eggs about 13 days before the emergence of the parasites. They do not appear to affect the parasites in any way and move about in the interior harmoniously with them.

Fresh spider eggs are a delicate pink and remain pale in color until they hatch, if not parasitized. However, when parasitized, the eggs assume a yellowish orange color within 12 days after being stung by the parasites and a few days before hatching the dark integument and blackish eyes of the parasites show clearly through the eggshells.

The complete life cycle of *Baeus californicus*, as determined at the Experiment Station, H. S. P. A. in Honolulu, averages about 22 days during the warm months of the year and from 25 to 29 days during winter. There is, however, a considerable amount of delayed emergence from every parasitized lot of spider eggs. The junior author has recorded the cycle data on over 32,000 parasites between September 1939, and April 1940, and the above averages are taken from his data. A sample case to indicate the extent of average delayed emergence that may occur follows: A fresh *mactans* egg sac was exposed to parasites on November 6, 1939. From this sac, parasites commenced emerging on November 30 and continued to come out almost daily until January 12, 1940, the bulk of the emergences occurring, however, during the first eight days.

Under field conditions in California, Dr. Pierce found that when spider eggs were parasitized at all, usually close to 100 per cent of them were so affected. The junior author collected a Black Widow spider egg sac at Lualualei, Oahu, on December 27, 1939, which had been attacked by parasites previously liberated by him in that locality. This sac produced 348 parasites and no spiders and is the first record of field work of the parasite in Hawaii.

The accompanying illustrations were prepared by W. Twigg-Smith.

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## Forms of Nitrogen for Sugar Cane

By R. J. BORDEN

Field experiments in which various forms of nitrogen, as originally applied from different nitrogen fertilizers, have been compared, have quite generally failed to indicate a superior efficiency for any one form over another upon the production of sugar cane or upon its quality; certainly it has been found questionable to generalize with respect to their comparative effectiveness.

Some of us believe that even if the different forms of nitrogen which are available in fertilizers for sugar cane should have different efficiencies, such a fact will be hard to establish in field tests, for the experimental error in field tests with cane will make it difficult to identify the expected small differences that are likely to be found as the real effects of the form of nitrogen applied. And yet if we could be assured that a specific form of nitrogen would be likely to result in even a very slight improvement in cane quality, even though it produced no more cane, we would welcome such assurance.

In designing this study\* to measure some of the effects from various forms of nitrogen, we were particularly concerned about furnishing our comparative treatments with similar conditions. Consequently we installed our study in Mitscherlich pots, located at Makiki, in such a way that all pots shared practically identical conditions of environment.

Identical amounts of three well-mixed soils from three different sources were separately potted. These soils may be characterized somewhat as follows:

Characteristics	(1) Manoa soil	(2) Makiki soil	(3) Ranch soil
Elevation . . . . .	550 ft.	40 ft.	80 ft.
Origin . . . . .	Residual	Alluvium	Alluvium
Color . . . . .	Yellow brown	Dull gray brown	Dull brown
Texture . . . . .	Light clay loam	Silty clay loam	Silty clay loam
Structure . . . . .	Crumb	Nut	Nut
Consistence . . . . .	Loose and friable	Plastic and sticky	Plastic and sticky
Volume weight . . . . .	.75	1.05	.90
Phosphate fixation . . . . .	90	30	45
pH . . . . .	5.7	7.0	7.3
% available N . . . . .	.0006	.0015	.0018
% available P <sub>2</sub> O <sub>5</sub> . . . . .	.010	.032	.007
% available K <sub>2</sub> O . . . . .	.003	.031	.012

Half of the pots with each soil were adequately fertilized with superphosphate; the other half received no phosphate. Nitrogen and potash, in adequate amounts for the type of culture proposed, were supplied similarly, except for the three forms of nitrogen which were being compared, i.e., ammoniacal nitrogen from ammonium sulphate, nitrate nitrogen from nitrate of soda, and synthetic non-proteid organic nitrogen from urea.

\* Project A-105—No. 122.

Single-eye cuttings of the cane variety 31-1389 were planted in these pots in July 1938. All growth thereafter was restricted to two primary stalks which started in each pot, so that stalk age would not introduce a variable factor at harvest. The crop was grown for 12 months and then harvested. All stalks were uniformly topped at the growing point and cut off at the surface of the soil.

Weights of millable stalks (lbs. cane) were secured and then all stalks were double milled (run through rollers twice) in a small 3-roller mill. The resulting bagasse was weighed and then dried along with the cane tops, and this combined dry weight added to the total weight of solids in the juice, and to the dry weight of cane leaves that had matured during the growth period, gave us our figures for total dry weight.

Samples of the crusher juice from each pot were then analyzed for Brix, pol, % N, % P<sub>2</sub>O<sub>5</sub>, and % K<sub>2</sub>O.

In the discussion which follows, we have attempted to point out the significant effects of the three nitrogen fertilizers upon the cane quality, as well as upon the total dry weights, millable cane, sugar, and the percentages of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O in the crusher juice. As was expected, many of these effects were considerably modified by interactions of the different forms of nitrogen with the other known differentials, hence both the soil and the phosphate fertilization are shown to affect the comparative efficiencies of the nitrogen carriers in some of the measurements that we made. We have discussed these interactions where we have found them to be significant.

#### *Yield % Cane—Cane Quality:*

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots		Avg. of 12 pots		
		without P <sub>2</sub> O <sub>5</sub>	with P <sub>2</sub> O <sub>5</sub>	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	13.31	13.08	13.55	13.22	13.33	13.40
Nitrate .....	13.21	13.34	13.08	13.15	13.32	13.18
Urea .....	13.73	13.61	13.85	13.70	13.82	13.68
Difference needed for odds of 19 to 1.....	.23	.32	.32	*	*	*
Difference needed for odds of 99 to 1.....	.31	.43	.43	*	*	*

\* = interactions are not significant.

Our primary objective in this study was to check upon the cane quality, as indicated by "yield % cane" calculations made from Brix and pol readings of the crusher juice from cane which had been fertilized with these specific salts. With the technique that could be used in our pot culture work, we were able to supply almost identical conditions of variety, source of seed, environment, soil, fertilization, irrigation, and stalk age at harvest, so that insofar as we are aware, our known applied differentials and their interactions were the only ones whose effects we measured.

The behavior of the form of nitrogen upon the cane quality was apparently not influenced by the soil. There is, however, evidence to show that the urea has produced the highest yield per cent cane, and that there was practically no difference between the effects of nitrate of soda and ammonium sulphate. This statement needs some modification, however, because there was apparently some interaction

with phosphate. Thus it would seem that when used with phosphate, the superiority of urea over ammonium sulphate was of doubtful significance, and that when used without phosphate, its gain over nitrate of soda was not so definite. It also appears that, when both were used with phosphate, the ammonium sulphate has produced a better cane quality than the nitrate of soda.

This is interesting and undoubtedly calls for further study and verification. At the same time, we recognize that it also is not the complete story, for we unfortunately cannot market our cane quality—we must have an answer in terms of recoverable sugar, and since this is rather dependent upon the amount of cane which is produced, we continue our analysis of the effects of these nitrogen carriers, first upon the cane yields, and thereafter upon the calculated sugar yields.

*Millable Cane (Lbs.):*

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots		Avg. of 12 pots		
		without $P_2O_5$	with $P_2O_5$	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	3.91	3.44	4.38	3.79	4.43	3.49
Nitrate .....	3.66	3.40	3.93	3.45	3.69	3.85
Urea .....	3.79	3.43	4.15	3.54	3.98	3.85
Difference needed for odds of 19 to 1 .....	.19	*	*	.33	.33	.33
Difference needed for odds of 99 to 1.....	.25	*	*	.44	.44	.44

\* = interactions are not significant.

The significant interactions, which were measured between the forms of nitrogen and the particular soils to which the nitrogen was supplied, make it difficult to generalize with reference to the effectiveness of any one form of nitrogen without specifying the soil upon which it will be used.

On the Manoa soil, ammonium sulphate was probably 10 per cent better than nitrate of soda but not better than urea. On the Makiki soil, ammonium sulphate produced 20 per cent more cane than nitrate of soda and 11 per cent more than urea. However, it is quite likely that both nitrate of soda and urea were 10 per cent more efficient than ammonium sulphate on the Ranch soil.

Although all three soils showed a response to phosphate, there was no indication that the efficiency of any form of nitrogen was significantly modified by this phosphate fertilization.

*Sugar Yields (Lbs.):*

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots		Avg. of 12 pots		
		without $P_2O_5$	with $P_2O_5$	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	.52	.45	.59	.51	.59	.47
Nitrate .....	.48	.45	.52	.45	.49	.51
Urea .....	.52	.47	.57	.49	.55	.53
Difference needed for odds of 19 to 1.....	.03	.04	.04	.05	.05	.05
Difference needed for odds of 99 to 1.....	.04	.06	.06	.07	.07	.07

Significant interactions between the main differential treatments, i.e., (a) the forms of nitrogen, (b) the fertilization with phosphate, and (c) the soil upon which the test was run, show that the efficiency of the three forms of nitrogen is not always the same when it comes to making sugar.

Without phosphate there was no favorite, but when phosphate was supplied, ammonium sulphate and perhaps urea also have produced 15 and 11 per cent more sugar respectively than nitrate of soda.

Soil has also modified the effectiveness of these nitrogen carriers, although the significance of the differences, except on the Makiki soil, does not warrant too great confidence in the amounts of the differences. On the Manoa soil, ammonium sulphate has probably produced 11 per cent more sugar than nitrate of soda. On the Makiki soil, ammonium sulphate has quite definitely produced 20 per cent more sugar than nitrate of soda, and urea was probably 12 per cent better than nitrate. However, on the Ranch soil, urea was apparently a 12 per cent better producer of sugar than ammonium sulphate.

Obviously we are cautioned by these results to avoid generalizations when we discuss the relative efficiencies of different forms of nitrogen fertilizers for sugar cane. Undoubtedly there are factors other than phosphates and soils, that may also interact differently with different sources of nitrogen and modify their relative effectiveness. Much research remains to be done before we can have all the facts to guide us.

#### *Total Dry Weight (Grams):*

In studies of this nature when an arbitrary pot technique is necessarily used, and the crop is harvested at a pre-determined age, it may happen that pertinent information is lost—information concerned with the potential or the ultimate cane and sugar yields which might have been obtained if the growth season had been extended. To secure some sort of an estimate of how this might affect our comparisons, we study the effects of the treatments upon the total dry weight, since this weight includes the cane tops and sucker growth that is still active at the time of our harvest.

In this present study, an analysis of the differences in the total dry weight is not significantly different from that of the millable cane; hence, we believe that the cane weights have quite reliably indicated the comparative status of the treatments at whatever age we might have selected for their harvest.

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots		Avg. of 12 pots		
		without $P_2O_5$	with $P_2O_5$	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	932	818	1046	883	1061	851
Nitrate .....	867	804	931	789	913	901
Urea .....	897	813	982	829	967	895
Difference needed for odds of 19 to 1.....	45	*	*	78	78	78
Difference needed for odds of 99 to 1.....	60	*	*	104	104	104

\* = interactions are not significant.

This total dry weight evidence would indicate (1) that the ammonium sulphate has produced more than nitrate of soda but not significantly more than urea; also that nitrate and urea were not significantly different in their effectiveness; (2) that it made no real difference upon their efficiency, whether or not phosphate was used with these three nitrogen carriers; and (3) that there was, however, an interaction of these forms of nitrogen with the soils upon which they were used, e.g., (a) although ammonium sulphate was probably superior to nitrate of soda on the Manoa soil and quite definitely so on the Makiki soil, it was not as effective on the Ranch soil; and (b) a likely superiority of ammonium sulphate over urea on the Makiki soil was not maintained on either of the other soils.

The difference in the effectiveness of these three forms of nitrogen on these soils may lie partly in the fact that they were quite different in their physical characteristics, especially their structure and consistency. The Manoa soil was the most open and porous, and its aeration and drainage were most ideal; both the Makiki and the Ranch soils were somewhat lacking in this desirable structure. Since there is no loss by leaching in the pots used in these studies, a loose open structure is not necessarily an undesirable feature. The upland Manoa soil is also known to be more abundantly supplied with organic matter than either of the drier lowland soils. Either we have failed to fully recognize such factors as causes of the different effects we have found, or there is an influence of some other soil component or condition other than an adequate supply of the ordinary nutrients, moisture, and such characteristics as we have measured, which has a real influence on the amount of vegetation that can be grown on a unit area of soil in a given period of time.

*Per Cent Total N in Crusher Juice:*

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots		Avg. of 12 pots		
		without $P_2O_5$	with $P_2O_5$	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	.043	.053	.032	.048	.034	.046
Nitrate .....	.032	.036	.029	.034	.032	.030
Urea .....	.026	.030	.023	.033	.024	.022
Difference needed for odds of 19 to 1.....	.003	*	*	*	*	*
Difference needed for odds of 99 to 1.....	.004	*	*	*	*	*

\* = interactions are not significant.

The comparative effect of the form of nitrogen upon the percentage of total nitrogen found in the crusher juice is apparently one that is not significantly influenced by the other factors which were included. However, it would appear that all three "forms" produced cane with less nitrogen in the juice when they were used in conjunction with phosphate than when phosphate was not supplied.

It is interesting to speculate as to whether the cane plant can actually take up more nitrogen from ammonium sulphate than from nitrate of soda or urea, or whether the higher percentage found in the juice means that it has tied up its nitrogen in more complex forms which have not been as completely broken down, to be either used or dissipated in the process of growth. Also, we wonder whether it is

possible that this greater nitrogen content of the juice from plants which received ammonium sulphate might be a reserve that was capable of being drawn upon for still further growth, if additional time had been allowed before the crop was harvested.

*Per Cent P<sub>2</sub>O<sub>5</sub> in Crusher Juice:*

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots—		Avg. of 12 pots—		
		without P <sub>2</sub> O <sub>5</sub>	with P <sub>2</sub> O <sub>5</sub>	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	.058	.038	.079	.016	.123	.035
Nitrate .....	.059	.036	.083	.028	.107	.044
Urea .....	.062	.039	.084	.021	.121	.043
Difference needed for odds of 19 to 1.....	*	*	*	.009	.009	.009
Difference needed for odds of 99 to 1.....	*	*	*	.012	.012	.012

\* = interactions are not significant.

On the Manoa soil the juice phosphate content was greater from cane fertilized with nitrate of soda than from cane which received ammonium sulphate, but on the Makiki soil, this condition was reversed—the ammonium sulphate, and urea also, both giving a juice with a higher per cent P<sub>2</sub>O<sub>5</sub> than nitrate. However, it can be shown that the interaction between the form of nitrogen and the soil, although significant, contributes a very small effect on the phosphate content of the crusher juice in comparison with the effect of the soil alone; apparently, the soils maintain their relative effects on the per cent P<sub>2</sub>O<sub>5</sub>, irrespective of the form of nitrogen used.

*Per Cent K<sub>2</sub>O in Crusher Juice:*

Form of nitrogen	Avg. of all 36 pots	Avg. of 18 pots—		Avg. of 12 pots—		
		without P <sub>2</sub> O <sub>5</sub>	with P <sub>2</sub> O <sub>5</sub>	on Manoa soil	on Makiki soil	on Ranch soil
Ammonia .....	.080	.091	.069	.050	.108	.083
Nitrate .....	.084	.086	.081	.060	.113	.080
Urea .....	.093	.098	.087	.057	.134	.088
Difference needed for odds of 19 to 1.....	.004	.007	.007	.008	.008	.008
Difference needed for odds of 99 to 1.....	.005	.009	.009	.011	.011	.011

As was the case with crusher juice phosphate, the soils had by far the greatest influence on the potash analyses also, but there are apparently many interactions which can affect the potash content of the crusher juice.

In general, the urea was responsible for a juice with more potash than either of the other forms of nitrogen; this was especially pronounced on the Makiki soil but was not so evident on the other two soils. On the Manoa soil, a higher percentage of potash was probably found in the juice from the nitrate of soda series than from the ammonium sulphate group.

Some interaction of the forms of nitrogen with phosphate is also indicated, but the effect of phosphates alone in significantly reducing the per cent K<sub>2</sub>O of the

crusher juice greatly overshadows its interaction with the form of nitrogen associated.

#### *Other Factors:*

Of incidental interest, because they were not a part of the primary objectives in this study, are (*a*) the comparisons between the three soils, and (*b*) the effects from the phosphate applications. These may be discussed briefly as follows:

*Total Dry Weight:* The Makiki soil produced 18 and 11 per cent more total dry weight than the Manoa and Ranch soils respectively.

A definite response to phosphate fertilization was secured on all three soils for the conditions under which they were cropped. This response was considerably greater on the Manoa and Ranch soils than on the Makiki soil—respectively 31 and 30 per cent greater as compared with 7 per cent.

*Cane Yields:* The yields of millable cane obtained from the Makiki soil were significantly greater than those from either the Manoa or the Ranch soil, but these two latter soils were not significantly different producers of millable cane.

Phosphate applications were beneficial on all three soils, though not to the same degree on the Makiki as on the other two soils.

*Yield % Cane:* Neither the soil nor the phosphate applications had any proven effect upon the cane quality as indicated by the yield per cent cane figures which we obtained.

*Sugar Yields:* Due to the higher cane yield, the Makiki soil produced 13 per cent more sugar than the Manoa soil and 8 per cent more than the Ranch soil. Similarly, because the phosphate fertilizer produced greater cane yields, especially on the Manoa and Ranch soils, it was responsible for 35 and 30 per cent increases in sugar respectively on these two soils. On the Makiki soil, the gain of 6 per cent cannot be reliably credited to the phosphate application.

*Per Cent N in Crusher Juice:* All three major factors of this experiment, namely the nitrogen, the soils, and the phosphate fertilization, have contributed to the percentage of nitrogen in the crusher juice. Cane grown on the Manoa soil had more nitrogen than that from either the Makiki or Ranch soils, which in turn were not significantly different in their influence. Phosphate applied to both the Manoa and Ranch soils resulted in significantly lower percentages of nitrogen in the juice; on the Makiki soil a similar effect was not significant. (It must not be overlooked that this higher *percentage* of nitrogen in the cane which had not received phosphate was associated with a smaller amount of cane and total dry weight that was produced without the phosphate fertilization; thus it may well be that the *total* amount of nitrogen in the plants was not significantly affected by the phosphate applications.)

*Per Cent P<sub>2</sub>O<sub>5</sub> in the Crusher Juice:* As was to be expected, the percentage of P<sub>2</sub>O<sub>5</sub> in the juice was dominated by the soil and by the phosphate fertilization. The Makiki soil put more phosphate into its cane than either of the others, and the Ranch soil furnished more than the Manoa soil. On all soils the effect of fertilizing with phosphate was a greatly increased content of P<sub>2</sub>O<sub>5</sub> in the juice.

*Per Cent K<sub>2</sub>O in the Crusher Juice:* The three soils had the same effects on the potash composition of the juice as they had on phosphate, i.e., the cane grown on

the Makiki soil had a significantly higher percentage of K<sub>2</sub>O than that from either of the other soils, and the Ranch soil apparently supplied more K<sub>2</sub>O than the Manoa soil. The effect due to phosphate alone is not significantly greater than the combined effect of phosphate and soils, and so it is not possible to generalize with reference to the effect of phosphate on the percentage of potash in cane from any specific soil. On the Manoa and Ranch soils, the potash composition was decreased when phosphate was supplied, but on the Makiki soil the per cent K<sub>2</sub>O was increased when phosphate was also given.

*Summary:*

Controlled studies in which three forms of nitrogen, as originally supplied in three different nitrogen fertilizers, have been compared for their effects upon several of the characteristics which are commonly measured in connection with experiments with sugar cane, have quite nicely pointed out some of the interacting factors which may influence the direct effects of the nitrogen fertilizer used. Hence, one should be exceedingly cautious not to generalize with respect to the relative efficiencies of nitrogen from commercial fertilizer sources.

Although the use of superphosphate with these nitrogen carriers did not significantly influence their comparative effects upon the cane yield or total dry weight, it apparently did modify their effects upon cane quality and sugar. Similarly, although the phosphate had little effect upon the comparative percentages of nitrogen and phosphate in the crusher juice of cane fertilized with these different forms of nitrogen, it did modify their relative effects upon the potash composition.

The respective effectiveness of the three nitrogen fertilizers upon the cane quality was not influenced by the soil upon which they were used. The soil, did, however, alter their comparative efficiencies as producers of cane and of sugar. At the same time, both the phosphate and potash juice comparisons from the three nitrogen sources were altered by soil differences, although the interaction of different soils with different forms of nitrogen had no significant influence on the percentage of nitrogen in the crusher juice.

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# The Synthesis of Sucrose by Excised Blades of Sugar Cane

## Time and Temperature

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By CONSTANCE E. HARTT

Evidence indicating that the leaf blade of the sugar cane plant can manufacture cane sugar when supplied with the simple sugars, glucose and fructose, was presented in a study of the synthesis of sucrose by excised blades of sugar cane (2). Numerous experiments have been conducted since the publication of that report, all of which show that the blade of the sugar cane plant has a particularly efficient mechanism for the formation of cane sugar from simple sugars. The questions of how soon the formation of sucrose can be detected, how long a detached leaf can continue to carry on the synthesis of sucrose, and how this process is affected by temperature, will be considered in this report.

### METHODS

All of the blades used in the experiments herein reported were of the variety H 109, obtained at the Experiment Station from border plantings in good soil which had received complete fertilization. As in the previous investigation, comparable blades were taken in all experiments. Counting the leaf with the highest visible dewlap as leaf number one, leaves numbers two and three were used in each test. Eight blades were used in each series, and one hundred cubic centimeters of solution were supplied to each blade.

With the exception of the temperature experiments, all of the tests were conducted in the constant-temperature room at  $27^{\circ}$  C.  $\pm 1^{\circ}$  C. The series held at low temperature were placed in the refrigerator. The constant-temperature rooms of the Enzyme Laboratory, the Sugar Technology Department, and the Pathology Department were used, all of which are thermostatically controlled. For the short temperature tests two ovens were used, but these were not as satisfactory as the constant-temperature rooms because they were too small.

The glucose used in these experiments was Bakers' Analyzed. The fructose was prepared from the rootstock of the ti by T. Tanimoto.

The methods of analysis of moisture, reducing sugars, and sucrose were the same as those previously described. In addition, residual fructose was determined iodometrically by the method of Hinton and Macara (3) as modified by Van der Plank (5). The difference between reducing sugars and residual fructose is reported as glucose.

### RESULTS

#### *Experiment 1. The hourly formation of cane sugar from glucose and fructose:*

In experiments 5 and 6 of the previous report (2), it was shown that in blades supplied with glucose or fructose, cane sugar fluctuated more than simple sugars

during the first 24 hours. Some sucrose was made the first hour, following which there was a lag of one or two hours in the formation of cane sugar, and then a steady increase in sucrose to the end of the first 24 hours. The suggestion was made that the lag in the formation of cane sugar after the first hour may have been due to the necessity for the formation of glucose from fructose and vice versa, since the blades were supplied with only one simple sugar and sucrose contains both. To determine this point, the following experiment was conducted:

The blades were freshly cut and placed in their respective solutions at 8:30 a.m., October 17, 1938. There were eight blades per series, with 100 cc. of 5 per cent glucose or fructose or 50 cc. of 5 per cent glucose + 50 cc. of 5 per cent fructose per blade. The series were as follows:

1. Untreated (sampled at 8:30 a.m., October 17, 1938).
2. Glucose 1 hour (sampled at 9:30 a.m.).
3. Fructose 1 hour (sampled at 9:30 a.m.).
4. Glucose + fructose 1 hour (sampled at 9:30 a.m.).
5. Glucose 2 hours (sampled at 10:30 a.m.).
6. Fructose 2 hours (sampled at 10:30 a.m.).
7. Glucose + fructose 2 hours (sampled at 10:30 a.m.).
8. Glucose 3 hours (sampled at 11:30 a.m.)
9. Fructose 3 hours (sampled at 11:30 a.m.).
10. Glucose + fructose 3 hours (sampled at 11:30 a.m.).
11. Glucose 5 hours (sampled at 1:30 p.m.).
12. Fructose 5 hours (sampled at 1:30 p.m.).
13. Glucose + fructose 5 hours (sampled at 1:30 p.m.).
14. Glucose 7 hours (sampled at 3:30 p.m.).
15. Fructose 7 hours (sampled at 3:30 p.m.).
16. Glucose + fructose 7 hours (sampled at 3:30 p.m.).
17. Glucose 11 hours (sampled at 7:30 p.m.).
18. Fructose 11 hours (sampled at 7:30 p.m.).
19. Glucose + fructose 11 hours (sampled at 7:30 p.m.).
20. Glucose 24 hours (sampled at 8:30 a.m., October 18, 1938).
21. Fructose 24 hours (sampled at 8:30 a.m.).
22. Glucose + fructose 24 hours (sampled at 8:30 a.m.).

The results of the determinations of moisture and sugars are presented in Tables I and II, and Fig. 1.

The total increase in sucrose for the 24-hour period in the blades supplied with glucose was 2.762 per cent upon the dry-weight basis; in the blades supplied with fructose, 2.884 per cent; and in the blades supplied with both, 3.305 per cent. Therefore, the blades which received both glucose and fructose made more cane sugar than those which received glucose or fructose alone.

Although the blades were supplied with glucose or fructose, their sucrose content changed more than their reducing sugar content. This is in agreement with the studies of the fluctuations of sugars in the blades of the sugar cane plant during the day and the night (1), and with results of the former synthesis tests (2). All of these results indicate that the simple sugars are fleeting intermediates in the leaf, while cane sugar is a storage product.

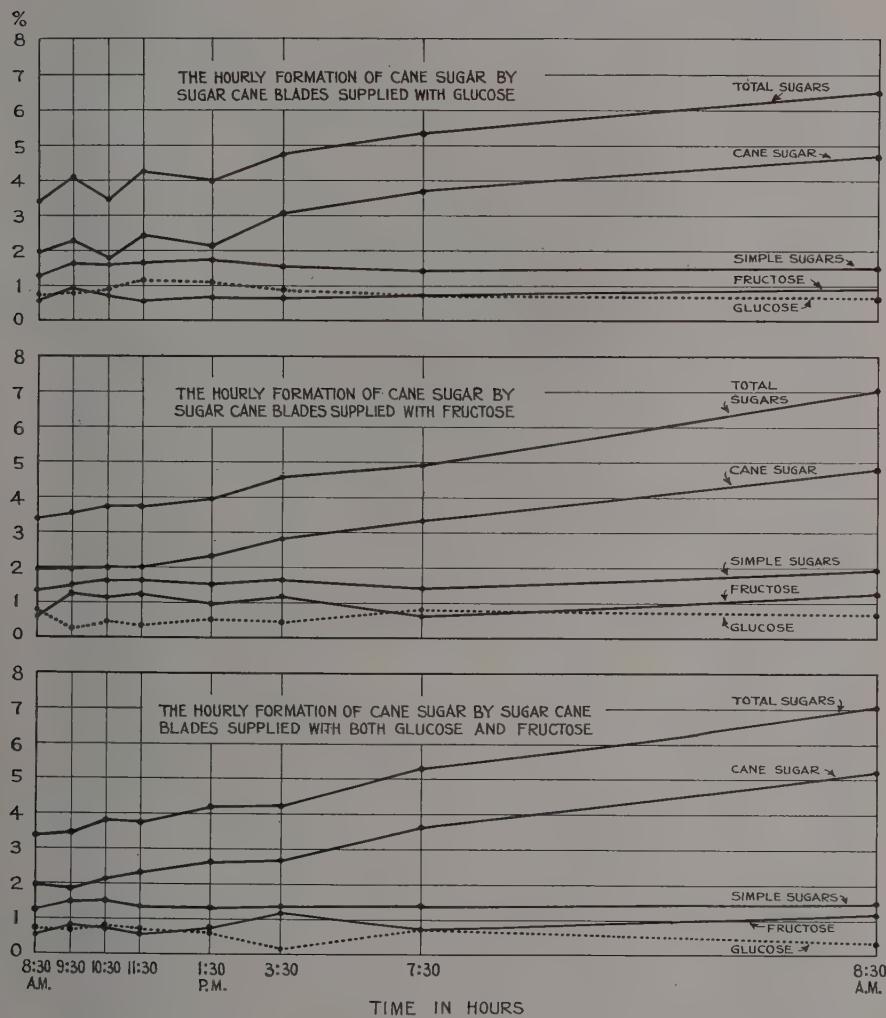


Fig. 1

The curves for fructose and glucose fluctuate a little more widely than the curves for the simple sugars as a whole. In the blades receiving only glucose there was more glucose than fructose during most of the first half of the experiment, but there was a tendency for fructose to gain over glucose by the end of the experiment. In the blades receiving only fructose, there was more fructose than glucose at almost every point on the curve. In the blades receiving both glucose and fructose, the percentages of glucose and fructose were equal for the first few hours, after which fructose gained.

In the blades given fructose alone, cane sugar began to increase during the fourth and fifth hours. In the blades given glucose alone, sucrose fluctuated widely the first few hours, the first significant increase occurring in the sixth and seventh

hours. In the blades given both glucose and fructose, sucrose increased significantly in the second and third hours.

The results of this experiment indicate that supplying blades with both glucose and fructose hastens and increases the formation of sucrose, compared with supplying only one of the simple sugars.

*Experiment 2. The daily formation of cane sugar from glucose and fructose:*

The object of this experiment was to find the "ceiling" or saturation point for sucrose formation in detached blades. Two tests have been conducted for this purpose, the first (2 A) lasting one week and the second (2 B) lasting two weeks.

TABLE I

MOISTURE AND SUGAR PERCENTAGES IN BLADES IN EXPERIMENT 1;  
MOISTURE PERCENTAGES EXPRESSED UPON THE WET-WEIGHT BASIS;  
SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	73.26 ± 0.095	1.296 ± 0.007	1.973 ± 0.008	3.374 ± 0.015
2. Glucose 1 hour . . . . .	73.47 ± 0.205	1.662 ± 0.002	2.247 ± 0.016	4.028 ± 0.019
3. Fructose 1 hour . . . . .	73.03 ± 0.029	1.453 . . . . .	1.967 . . . . .	3.524 . . . . .
4. Both 1 hour . . . . .	73.63 ± 0.024	1.514 ± 0.007	1.860 ± 0.004	3.472 ± 0.011
5. Glucose 2 hours . . . . .	73.77 ± 0.091	1.589 ± 0.019	1.735 ± 0.016	3.416 ± 0.002
6. Fructose 2 hours . . . . .	73.59 ± 0.071	1.611 ± 0.005	2.008 ± 0.002	3.725 ± 0.007
7. Both 2 hours . . . . .	73.49 ± 0.038	1.556 ± 0.012	2.143 ± 0.013	3.812 ± 0.001
8. Glucose 3 hours . . . . .	73.38 ± 0.038	1.645 ± 0.005	2.425 ± 0.009	4.208 ± 0.015
9. Fructose 3 hours . . . . .	73.97 ± 0.172	1.618 ± 0.008	2.021 ± 0.003	3.746 ± 0.011
10. Both 3 hours . . . . .	72.59 ± 0.105	1.322 ± 0.015	2.340 ± 0.003	3.786 ± 0.007
11. Glucose 5 hours . . . . .	74.41 ± 0.119	1.730 ± 0.012	2.115 ± 0.003	3.957 ± 0.015
12. Fructose 5 hours . . . . .	72.65 ± 0.019	1.507 ± 0.008	2.319 ± 0.001	3.949 ± 0.009
13. Both 5 hours . . . . .	72.19 ± 0.091	1.367 ± 0.016	2.678 ± 0.017	4.186 ± 0.002
14. Glucose 7 hours . . . . .	73.56 ± 0.043	1.533 ± 0.006	3.023 ± 0.012	4.715 ± 0.006
15. Fructose 7 hours . . . . .	72.79 ± 0.067	1.639 ± 0.007	2.808 ± 0.010	4.595 ± 0.003
16. Both 7 hours . . . . .	72.50 ± 0.114	1.387 ± 0.001	2.698 ± 0.005	4.228 ± 0.006
17. Glucose 11 hours . . . . .	72.04 ± 0.205	1.455 ± 0.016	3.677 ± 0.015	5.326 ± 0.032
18. Fructose 11 hours . . . . .	72.40 ± 0.014	1.412 ± 0.003	3.350 ± 0.008	4.938 ± 0.012
19. Both 11 hours . . . . .	72.21 ± 0.052	1.472 ± 0.007	3.671 ± 0.005	5.337 ± 0.013
20. Glucose 24 hours . . . . .	72.74 ± 0.019	1.575 ± 0.002	4.735 ± 0.004	6.560 ± 0.002
21. Fructose 24 hours . . . . .	72.99 ± 0.191	1.947 ± 0.005	4.857 ± 0.011	7.059 ± 0.006
22. Both 24 hours . . . . .	72.02 ± 0.052	1.515 ± 0.001	5.278 ± 0.021	7.071 ± 0.024

TABLE II

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES IN EXPERIMENT 1,  
EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated .....	0.583 ± 0.032	0.713 ± 0.039
2. Glucose 1 hour.....	0.879 ± 0.025	0.783 ± 0.028
3. Fructose 1 hour.....	1.205 ....	0.248 ....
4. Both 1 hour.....	0.794 ± 0.003	0.720 ± 0.004
5. Glucose 2 hours.....	0.699 ± 0.007	0.890 ± 0.012
6. Fructose 2 hours.....	1.136 ± 0.001	0.474 ± 0.006
7. Both 2 hours.....	0.756 ± 0.027	0.799 ± 0.014
8. Glucose 3 hours.....	0.527 ± 0.001	1.117 ± 0.003
9. Fructose 3 hours.....	1.246 ± 0.035	0.372 ± 0.042
10. Both 3 hours.....	0.590 ....	0.702 ....
11. Glucose 5 hours.....	0.654 ± 0.018	1.076 ± 0.007
12. Fructose 5 hours.....	0.961 ± 0.004	0.546 ± 0.004
13. Both 5 hours.....	0.721 ± 0.019	0.645 ± 0.003
14. Glucose 7 hours.....	0.681 ± 0.038	0.851 ± 0.043
15. Fructose 7 hours.....	1.189 ± 0.043	0.449 ± 0.036
16. Both 7 hours.....	1.214 ± 0.006	0.173 ± 0.005
17. Glucose 11 hours.....	0.698 ± 0.019	0.757 ± 0.003
18. Fructose 11 hours.....	0.618 ± 0.016	0.794 ± 0.013
19. Both 11 hours.....	0.733 ± 0.006	0.738 ± 0.001
20. Glucose 24 hours.....	0.904 ± 0.238	0.671 ± 0.026
21. Fructose 24 hours.....	1.276 ± 0.021	0.671 ± 0.026
22. Both 24 hours.....	1.197 ± 0.044	0.318 ± 0.045

In Experiment 2 A, the blades were placed in 5 per cent glucose in the dark at room temperature on the morning of July 25, 1938. The glucose was renewed daily and at the same time the flasks and the bases of the blades were washed. The blades were green and turgid until the third day, when the tips of several blades were yellow but still turgid. On the fourth day, the blades were more yellow and a little dried at the tips. On the seventh day, the blades were still more yellow and more dried at the tips, but alive.

The series were as follows:

1. Untreated.
2. Sampled after 1 day.
3. Sampled after 2 days.
4. Sampled after 3 days.
5. Sampled after 4 days.
6. Sampled after 7 days.

The results of the determinations of moisture and sugars are presented in Tables III and IV and Fig. 2.

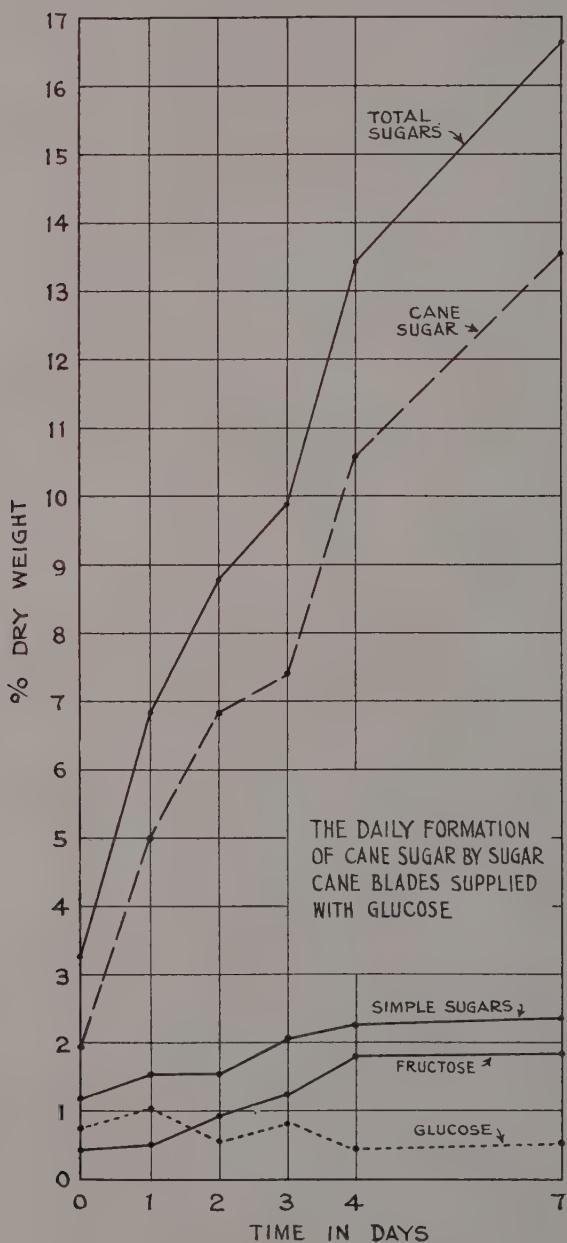


Fig. 2

TABLE III

MOISTURE AND SUGAR PERCENTAGES IN BLADES IN EXPERIMENT 2 A;  
MOISTURE PERCENTAGES EXPRESSED UPON THE WET-WEIGHT BASIS;  
SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	72.60 ± 0.090	1.186 ± 0.004	1.948 ± 0.008	3.236 ± 0.013
2. 1 day . . . . .	71.92 ± 0.186	1.563 ± 0.004	4.996 ± 0.004	6.823 ± 0.000
3. 2 days . . . . .	70.97 ± 0.029	1.564 ± 0.005	6.854 ± 0.002	8.780 ± 0.003
4. 3 days . . . . .	68.67 ± 0.157	2.045 ± 0.001	7.413 ± 0.051	9.849 ± 0.052
5. 4 days . . . . .	68.44 ± 0.095	2.273 ± 0.005	10.585 ± 0.001	13.415 ± 0.007
6. 7 days . . . . .	63.86 ± 0.062	2.380 ± 0.014	13.589 ± 0.108	16.684 ± 0.099

TABLE IV

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES IN EXPERIMENT 2 A,  
EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	0.413 ± 0.040	0.772 ± 0.045
2. 1 day . . . . .	0.507 ± 0.018	1.056 ± 0.022
3. 2 days . . . . .	0.984 ± 0.004	0.580 ± 0.000
4. 3 days . . . . .	1.218 ± 0.023	0.827 ± 0.022
5. 4 days . . . . .	1.811 ± 0.008	0.462 ± 0.003
6. 7 days . . . . .	1.832 ± 0.030	0.548 ± 0.044

The results for sucrose in Experiment 2 A were recalculated on the following bases: original fresh weight, final wet weight, sugar-free dry weight, and grams sucrose per 100 grams water, and are so presented in Table V, which shows large increases in percentages of sucrose by every method of calculation.

TABLE V

CANE SUGAR PERCENTAGES IN BLADES IN EXPERIMENT 2 A, COMPARING  
METHODS OF CALCULATION

Series	Original fresh weight	Wet weight	Grams sucrose per 100 grams water	Sugar-free dry weight
1. Untreated . . . . .	0.534	0.534	0.735	2.209
2. 1 day . . . . .	1.389	1.402	1.950	6.598
3. 2 days . . . . .	1.945	1.989	2.803	9.825
4. 3 days . . . . .	2.197	2.322	3.406	10.814
5. 4 days . . . . .	3.149	3.339	4.879	18.405
6. 7 days . . . . .	4.315	4.911	7.691	25.236

Table III shows that the blades lost moisture steadily during the week. They increased in simple sugars and particularly in cane sugar. Although the percentage of cane sugar was increased many times during the week by every method of calculation (Table V), there is no indication that the saturation point for sucrose was reached.

These blades were all supplied with glucose but no fructose. Yet fructose accumulated and glucose showed only minor fluctuations. Evidently the conversion of glucose to fructose took place so rapidly that not all of the fructose formed could be made into sucrose.

Because the maximum sucrose content of the detached blades in Experiment 2 A was not reached in one week, Experiment 2 B was conducted. The blades were cut in the afternoon of September 6, 1938, and placed in tap water in the dark overnight. They were transferred to 5 per cent glucose the following day at 9 a.m. The glucose was renewed daily, the flasks and the blades being washed, and the ends of the blades trimmed at the same time.

Yellowing of the tips of the blades was first noted on the third day. By the fifth day most of the blades were yellow at the tip, but the blades were all still turgid. On the sixth day it was noted that the yellowing was progressing downward; those blades with the largest yellow area were then drying at the tip, the dried area being smaller than the yellow area. Some of the blades had been torn a little during handling. On the seventh day all the blades were yellow at the tip, in varying degree; in many leaves the yellow area progressed downward along the midrib, in some around the torn places, and in some along the edges. On the ninth day the blades had yellow areas right down to the base, but were not completely yellow. On the twelfth day the blades were all in bad condition, yellow, dried at the tips, but not completely yellow. On the fourteenth or final day, the blades were in very bad condition, almost completely yellow, very dry at the tip and about half the length of the leaf.

A narrow dark discoloration along the cut end of the blade was apparent daily and removed daily. Every day the glucose was examined before it was thrown away. It was never cloudy or ill smelling.

Notwithstanding the yellowed and dried condition of the blades, the entire blades were ground, mixed thoroughly, and sampled each day.

The series were as follows:

1. Untreated.
2. Sampled after 1 day.
3. Sampled after 2 days.
4. Sampled after 5 days.
5. Sampled after 6 days.
6. Sampled after 7 days.
7. Sampled after 8 days.
8. Sampled after 9 days.
9. Sampled after 10 days.
10. Sampled after 11 days.
11. Sampled after 12 days.
12. Sampled after 13 days.
13. Sampled after 14 days.

The results of the moisture and sugar determinations are recorded in Tables VI and VII and Fig. 3.

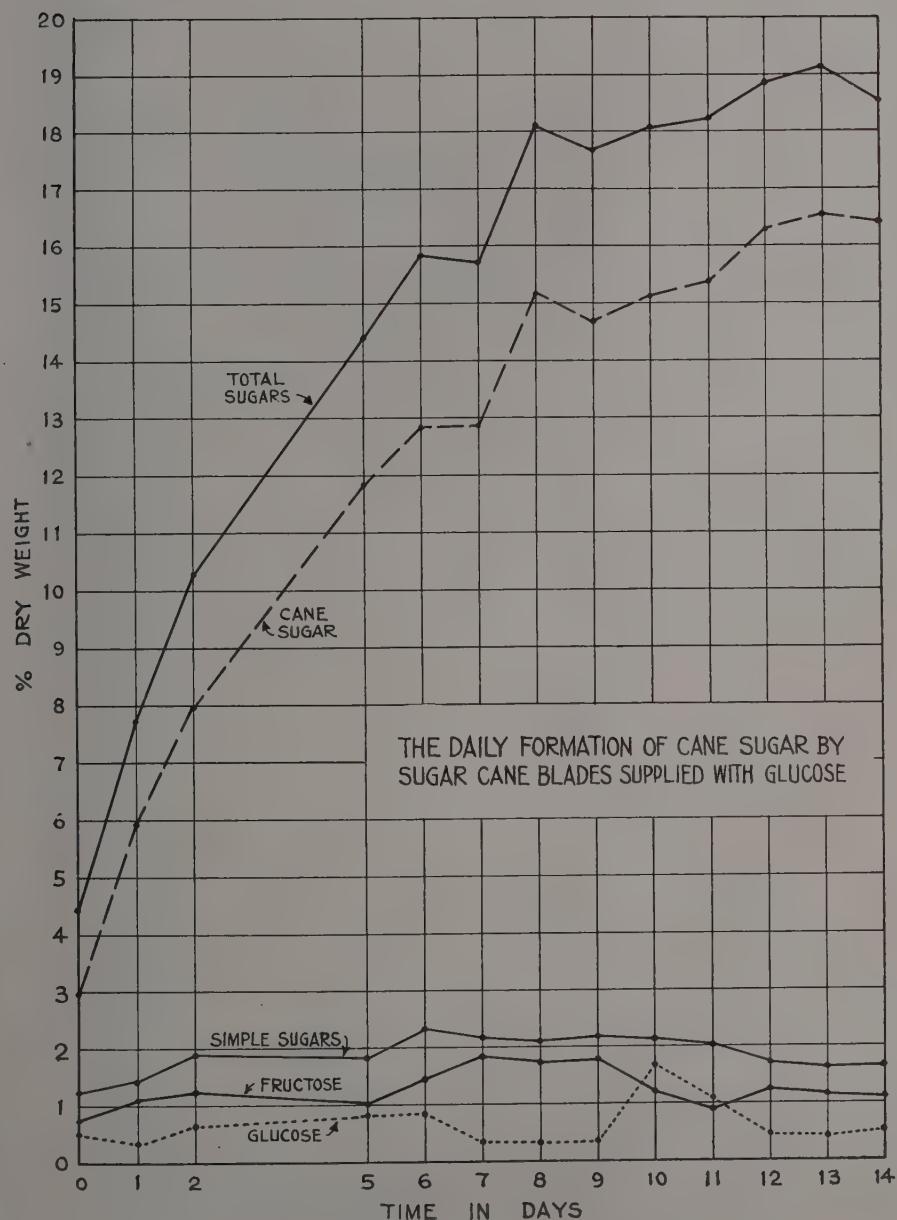


Fig. 3

TABLE VI

MOISTURE AND SUGAR PERCENTAGES IN BLADES IN EXPERIMENT 2 B:  
MOISTURE PERCENTAGES EXPRESSED UPON THE WET-WEIGHT BASIS;  
SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	70.30 ± 0.029	1.268 ± 0.027	2.974 ± 0.022	4.399 ± 0.003
2. 1 day . . . . .	70.06 ± 0.067	1.465 ± 0.019	5.940 ± 0.008	7.718 ± 0.028
3. 2 days . . . . .	68.02 ± 0.052	1.915 ± 0.005	7.952 ± 0.027	10.286 ± 0.024
4. 5 days . . . . .	64.87 ± 0.071	1.863 ± 0.001	11.849 ± 0.054	14.337 ± 0.059
5. 6 days . . . . .	64.67 ± 0.033	2.338 ± 0.004	12.807 ± 0.003	15.819 ± 0.001
6. 7 days . . . . .	64.98 ± 0.052	2.188 ± 0.007	12.830 ± 0.052	15.693 ± 0.061
7. 8 days . . . . .	63.17 ± 0.000	2.111 ± 0.026	15.164 ± 0.006	18.073 ± 0.032
8. 9 days . . . . .	61.04 ± 0.024	2.206 ± 0.013	14.685 ± 0.048	17.664 ± 0.037
9. 10 days . . . . .	62.36 ± 0.167	2.183 ± 0.034	15.106 ± 0.029	18.084 ± 0.064
10. 11 days . . . . .	59.27 ± 0.234	2.049 ± 0.029	15.365 ± 0.041	18.233 ± 0.072
11. 12 days . . . . .	57.86 ± 0.000	1.739 ± 0.017	16.270 ± 0.000	18.865 ± 0.018
12. 13 days . . . . .	56.92 ± 0.296	1.648 ± 0.037	16.585 ± 0.062	19.107 ± 0.103
13. 14 days . . . . .	57.71 ± 0.205	1.700 ± 0.023	16.430 ± 0.162	18.554 ± 0.017

Table VI and Fig. 3 show that the blades decreased in percentage of moisture to the end of the experiment. The percentage of cane sugar increased rapidly for eight days, after which time the rate of increase was less. There was a slight increase in percentage of simple sugars during the first six days, followed by a decrease. The percentages of glucose and fructose fluctuated, with fructose generally higher than glucose, although glucose was the sugar supplied.

TABLE VII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES IN EXPERIMENT 2 B,  
EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	0.750 ± 0.013	0.518 ± 0.014
2. 1 day . . . . .	1.110 ± 0.006	0.354 ± 0.024
3. 2 days . . . . .	1.241 ± 0.000	0.673 ± 0.004
4. 5 days . . . . .	1.022 ± 0.002	0.841 ± 0.003
5. 6 days . . . . .	1.472 ± 0.000	0.866 ± 0.003
6. 7 days . . . . .	1.861 ± 0.008	0.326 ± 0.001
7. 8 days . . . . .	1.785 ± 0.013	0.325 ± 0.012
8. 9 days . . . . .	1.815 ± 0.004	0.391 ± 0.018
9. 10 days . . . . .	1.217 ± 0.025	1.725 ± 0.024
10. 11 days . . . . .	0.926 ± 0.000	1.123 ± 0.028
11. 12 days . . . . .	1.268 ± 0.017	0.471 ± 0.000
12. 13 days . . . . .	1.203 ± 0.007	0.445 ± 0.044
13. 14 days . . . . .	1.133 ± 0.034	0.567 ± 0.010

The results of Experiment 2 A, 2 B, (at room temperature) and 4 A, 4 B, and 4 C (at 30° C.) were averaged and then smoothed by the method of moving averages. The smoothed curves are presented in Fig. 4, in which the results for the second week are entirely from Experiment 2 B, since the other experiments were shorter in duration. Fig. 4 shows that even after two weeks in 5 per cent glucose, the blades were still able to manufacture sucrose. It would seem that death of the leaf occurs before the mechanism for the manufacture of sucrose is destroyed.

The steepest portion of the sucrose curve is that for the first three or four

days. This period was therefore chosen for the studies of time-temperature relationships in Experiment 4.

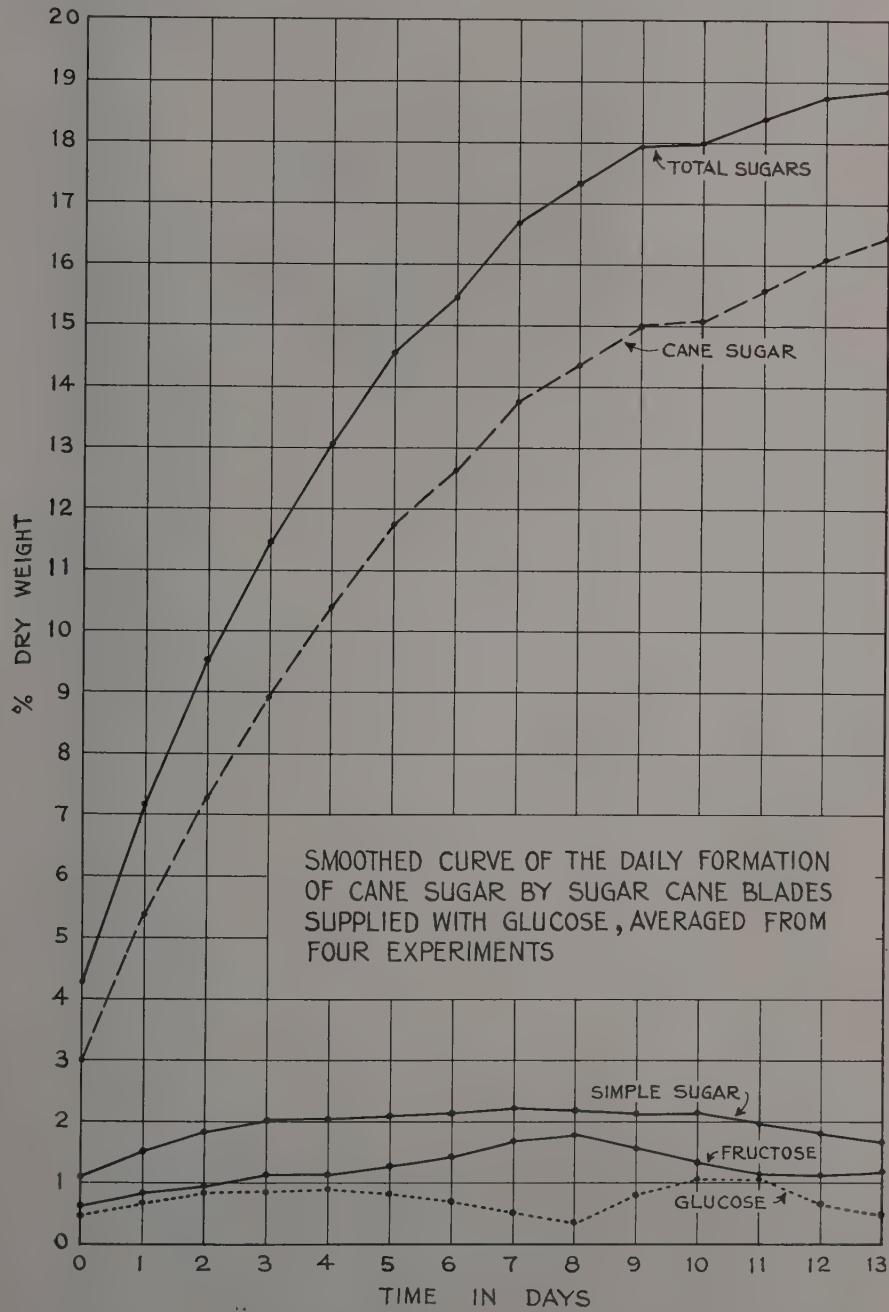


Fig. 4

*Experiment 3. The effect of temperature upon the formation of sucrose from glucose and fructose:*

Three tests have been conducted in which blades supplied with simple sugar were kept at different temperatures for 24 hours. In Experiment 3 A, blades supplied with both glucose and fructose (5 per cent reducing sugars) at pH 4.6 were kept at the following temperatures: 6°, 19°, 31°, 39.6°, and 50° C. In this experiment the series at 31° C. was in too small an oven. In Experiment 3 B, blades supplied with both glucose and fructose were kept at the following temperatures: 7.5°, 19°, 30.5°, and 40.8° C. In Experiment 3 C, blades supplied with 5 per cent glucose were kept at the following temperatures: 7.5°, 19°, 40.2°, and 50.5° C.

In Experiment 3 A, after 24 hours, the blades which had been kept at 6°, 19°, and 31° C. were in uniformly good condition, appearing exactly as at the start of the test. The blades which had been kept at 40° C. were dry at the tip and nearly dry for the outer half, but they were not discolored. The blades which had been kept at 50° C. were nearly dry throughout their entire length, and were grayish green in color.

The results of the determinations of moisture and sugars in Experiment 3 A are recorded in Tables VIII and IX.

TABLE VIII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE AND FRUCTOSE IN EXPERIMENT 3 A; MOISTURE PERCENTAGES UPON THE WET-WEIGHT BASIS; SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	73.89 ± 0.229	0.863 ± 0.007	2.436 ± 0.001	3.428 ± 0.024
2. 6° C. . . . .	71.76 ± 0.057	4.586 ± 0.022	2.076 ± 0.010	6.772 ± 0.009
3. 19° C. . . . .	72.99 ± 0.119	1.584 ± 0.029	5.158 ± 0.052	6.964 ± 0.060
4. 31° C. . . . .	71.92 ± 0.167	0.854 ± 0.036	3.353 ± 0.016	4.383 ± 0.019
5. 39.6° C. . . . .	62.55 ± 0.119	8.973 . . . . .	8.020 . . . . .	17.410 . . . . .
6. 50° C. . . . .	47.64 ± 0.281	19.180 ± 0.024	3.040 ± 0.029	22.390 ± 0.000

TABLE IX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE AND FRUCTOSE IN EXPERIMENT 3 A, EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	0.379 ± 0.037	0.484 ± 0.030
2. 6° C. . . . .	2.555 ± 0.001	2.031 ± 0.020
3. 19° C. . . . .	0.820 ± 0.084	0.714 ± 0.090
4. 31° C. . . . .	0.046 ± 0.012	0.808 ± 0.048
5. 39.6° C. . . . .	5.086 . . . . .	3.887 . . . . .
6. 50° C. . . . .	15.810 ± 0.091	3.370 ± 0.062

Table VIII shows that the percentage of moisture in the blades was not much affected by temperatures from 6°–31° C., but was decreased by the higher temperatures. The percentage of simple sugars increased more at 6° C. than at 19° C. or at 31° C. It is evident that no formation of sucrose took place at 6° C. With the exception of the series at 31° C., which was in too small an oven and hence

did not absorb as much sugar as the other series, the formation of cane sugar was increased by temperature from approximately 20° to 40° C. Very little formation of sucrose took place at 50° C.

Table IX shows that the percentages of fructose and glucose were approximately equal in the untreated series. All of the treated series were given equal parts of glucose and fructose, yet, with the exception of series 4 (31° C.), they all contained more fructose than glucose. At 39.6° C. there was an accumulation of both fructose and glucose, indicating that absorption took place more rapidly than synthesis. At 39.6° C. there was a slightly greater accumulation of fructose than of glucose, and at 50° C. there was a considerably greater accumulation of fructose than of glucose. Although all series began with equal amounts of glucose and fructose, and all received equal amounts of both, yet at 50° C. there was five times as much fructose as glucose. Thus the conversion of glucose to fructose was decidedly favored by high temperature.

In Experiment 3 B, blades were given equal parts of glucose and fructose (5 per cent), unbuffered, and kept at the following temperatures for 24 hours: 7.5°, 19°, 30.5°, and 40.8° C. The small oven was not used. The percentages of moisture and sugars and the activity of invertase are recorded in Tables X and XI.

TABLE X

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE AND FRUCTOSE IN EXPERIMENT 3 B; MOISTURE PERCENTAGES UPON THE WET-WEIGHT BASIS; SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	74.83 ± 0.000	1.225 ± 0.014	1.848 ± 0.008	3.171 ± 0.022
2. 7.5° C. . . . .	73.37 ± 0.076	4.203 ± 0.018	2.587 ± 0.024	6.858 ± 0.026
3. 19° C. . . . .	73.35 ± 0.062	1.646 ± 0.026	4.076 ± 0.029	5.937 ± 0.005
4. 30.5° C. . . . .	72.37 ± 0.067	1.497 ± 0.023	5.636 ± 0.069	7.306 ± 0.037
5. 40.8° C. . . . .	60.11 ± 0.224	7.316 ± 0.042	9.090 ± 0.038	16.880 ± 0.000

TABLE XI

FRUCTOSE AND GLUCOSE PERCENTAGES AND INVERTASE ACTIVITY IN BLADES IN EXPERIMENT 3 B; SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS; INVERTASE ACTIVITY EXPRESSED AS CC. N/20 KMnO<sub>4</sub>

Series	Fructose	Glucose	Invertase
1. Untreated . . . . .	0.612 ± 0.007	0.613 ± 0.007	13.80
2. 7.5° C. . . . .	2.183 ± 0.014	2.020 ± 0.004	12.79
3. 19° C. . . . .	1.109 ± 0.070	0.537 ± 0.042	15.70
4. 30.5° C. . . . .	1.163 ± 0.003	0.334 ± 0.027	14.11
5. 40.8° C. . . . .	4.527 ± 0.099	2.788 ± 0.141	18.45

The results of Experiment 3 B agree with those of Experiment 3 A. Tables X and XI show that absorption of sugar took place as well at 7.5° C. as at 19° and 30.5° C., but at the lowest temperature both fructose and glucose accumulated due to the small formation of sucrose. The percentages of cane sugar were positively correlated with temperature from 7.5° to 40.8° C. More fructose than glucose accumulated at 40.8° C.

The activity of invertase was greatest in the blades which had been kept at 40.8° C. This was not the direct effect of the increase in sucrose, because that was eliminated by the controls.

In Experiment 3 C, blades given 5 per cent glucose were kept at 7.5°, 19°, 40.2°, and 50.5° C. After 24 hours, the blades at 7.5° and 19° C. were as green and turgid as at the start, but the blades at 40.2° C. were somewhat dried at the tips and the blades at 50.5° C. were very dry. The results of the moisture and sugar determinations are presented in Tables XII and XIII.

TABLE XII

MOISTURE AND SUGAR PERCENTAGES IN BLADES GIVEN 5 PER CENT GLUCOSE IN EXPERIMENT 3 C; MOISTURE PERCENTAGES UPON THE WET-WEIGHT BASIS, SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	71.92 ± 0.024	1.245 ± 0.022	2.198 ± 0.022	3.558 ± 0.001
2. 7.5° C. . . . .	72.05 ± 0.057	5.212 ± 0.001	2.498 ± 0.005	7.842 ± 0.006
3. 19° C. . . . .	72.28 ± 0.038	2.265 ± 0.036	5.213 ± 0.048	7.753 ± 0.014
4. 40.2° C. . . . .	66.96 ± 0.186	7.466 ± 0.064	14.060 ± 0.057	22.260 ± 0.000
5. 50.5° C. . . . .	35.44 ± 0.396	12.930 . . . . .	7.310 . . . . .	20.240 . . . . .

TABLE XIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN EXPERIMENT 3 C, EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	1.001 ± 0.007	0.244 ± 0.015
2. 7.5° C. . . . .	1.115 ± 0.082	4.097 ± 0.083
3. 19° C. . . . .	1.402 ± 0.033	0.863 ± 0.069
4. 40.2° C. . . . .	2.876 ± 0.029	4.589 ± 0.035
5. 50.5° C. . . . .	2.834 . . . . .	10.100 . . . . .

Table XII shows that the percentage of moisture was the same at 7.5° and 19° C., but was lower at 40.2° C. and very much lower at 50.5° C. Glucose, but not fructose, accumulated at 7.5° C. and very little cane sugar was made at that temperature. The percentage of cane sugar increased from 7.5° to 40.2° C., but decreased at 50.5° C.

The results of the determinations of cane sugar obtained in Experiments 3 B and 3 C, recalculated as grams sucrose per 100 grams water, are recorded in Table XIV.

TABLE XIV

GRAMS SUCROSE PER 100 GRAMS WATER IN EXPERIMENT 3 B AND 3 C

Treatment	Experiment	
	3 B	3 C
Untreated . . . . .	0.614	0.850
7.5° C. . . . .	0.914	0.968
19° C. . . . .	1.480	1.995
30.5° C. . . . .	2.105	...
40.2 or 40.8° C. . . . .	6.033	6.936
50.5° C. . . . .	13.318	

If the percentage of cane sugar in the untreated series is subtracted from that in the treated series, a measure of the amount of sucrose formed in 24 hours at the different temperatures is obtained. This was done for Experiments 3 A, 3 B, and 3 C, the results were averaged, and they are presented in Table XV and Fig. 5. The percentage of glucose absorbed that is converted into sucrose is called the "synthetic efficiency." The averages of these figures for the three experiments are reported in Table XV.

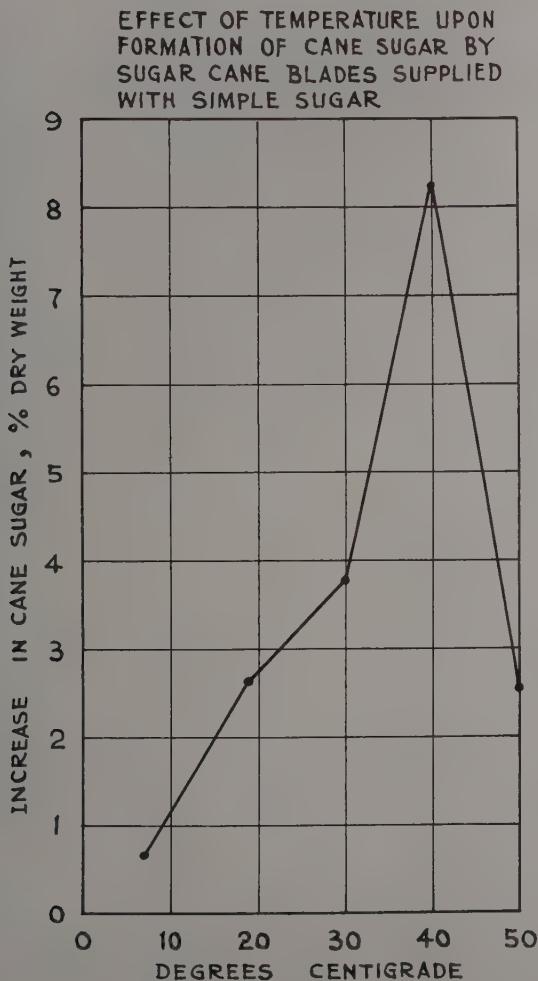


Fig. 5

TABLE XV

THE INCREASE IN SUCROSE AND THE SYNTHETIC EFFICIENCIES, AVERAGED FOR EXPERIMENTS 3 A, 3 B AND 3 C

Temperature	Increase in sucrose	Synthetic efficiency
6-7.5° C. ....	0.679	9.01
19° C. ....	2.655	76.46
30° C. ....	3.788	93.81
40° C. ....	8.230	52.04
50° C. ....	2.580	16.97

While it is true that more sucrose was made at 40° than at 30° C., as shown by the actual increase in sucrose, yet the mechanism of synthesis, whatever it is, operated most efficiently at 30° C., as shown by the synthetic efficiency.

*Experiment 4. Time, temperature, and the formation of sucrose from glucose or fructose:*

The object of this experiment was to determine the effect of temperature over a period of days, upon the interconversion of glucose and fructose and the formation of sucrose. Because Experiment 2 showed that the most rapid formation of sucrose took place in the first few days, that was the period of time selected for Experiment 4. Because Experiment 3 showed that keeping blades at 50° C. for one day dried them severely, and because almost no sucrose was formed at that temperature, it was omitted in Experiment 4. Three tests have been conducted, two in which glucose was used and one in which fructose was used.

In Experiment 4 A, blades given 5 per cent glucose were kept four days at the following temperatures: 6°, 20°, 30°, and 39.1° C. The glucose was renewed daily, the flasks being washed and the ends of the blades washed and trimmed at the same time. The results of the moisture and sugar determinations are reported in Tables XVI and XVII and Fig. 6.

Tables XVI and XVII and Fig. 6 show that at 6° C. the conversion of glucose to fructose was greatly retarded. Only a little sucrose was formed. The absorption of glucose took place readily, but most of the glucose absorbed remained as glucose in the blade.

At 20° C. the conversion of glucose to fructose took place as quickly as the glucose was absorbed. Considerable sucrose was formed. The absorption of glucose as shown by the total sugar curve seemed no faster than at 6° C. Most of the glucose absorbed was converted into sucrose.

At 30° C. the conversion of glucose to fructose was accelerated, so that there was more fructose than glucose in the blade. More glucose was absorbed and more sucrose formed than at 20° C.

At 40° C. more glucose was absorbed, more glucose was converted into fructose, and more sucrose was made than at any other temperature.

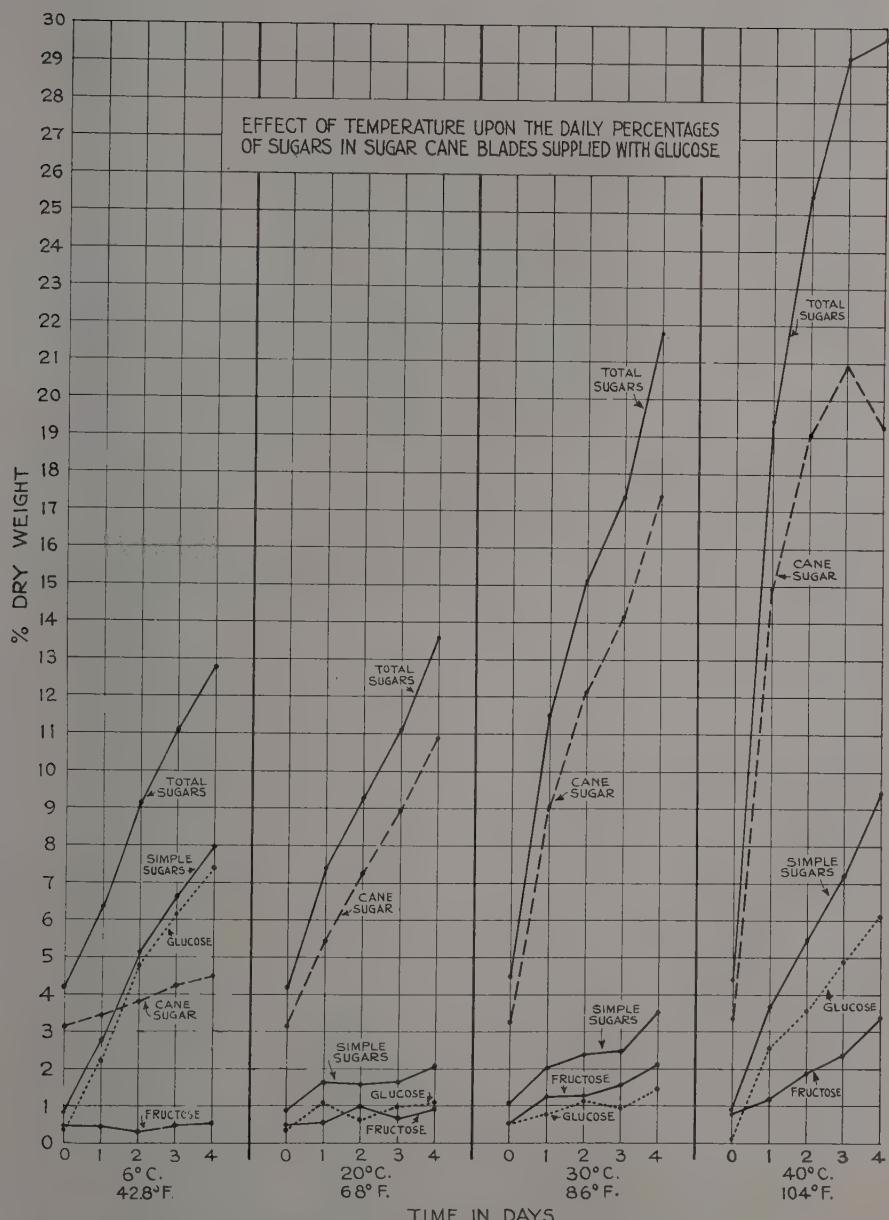


Fig. 6

TABLE XVI

MOISTURE AND SUGAR PERCENTAGES IN BLADES GIVEN GLUCOSE IN EXPERIMENT 4 A; MOISTURE PERCENTAGES UPON THE WET-WEIGHT BASIS; SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	68.32 ± 0.014	0.860 ± 0.007	3.173 ± 0.006	4.200 ± 0.000
2. 6°—1 day . . . . .	67.85 ± 0.043	2.717 ± 0.031	3.452 ± 0.009	6.351 ± 0.041
3. 6°—2 days . . . . .	65.14 ± 0.145	5.116 ± 0.018	3.807 ± 0.006	9.124 ± 0.011
4. 6°—3 days . . . . .	65.28 ± 0.009	6.615 ± 0.011	4.254 ± 0.014	11.093 ± 0.003
5. 6°—4 days . . . . .	65.53 ± 0.114	7.992 ± 0.036	4.518 ± 0.000	12.748 ± 0.036
6. 20°—1 day . . . . .	67.47 ± 0.110	1.634 ± 0.005	5.448 ± 0.004	7.368 ± 0.001
7. 20°—2 days . . . . .	65.94 ± 0.195	1.601 ± 0.031	7.264 ± 0.033	9.248 ± 0.003
8. 20°—3 days . . . . .	64.97 ± 0.033	1.660 ± 0.002	8.939 ± 0.037	11.069 ± 0.041
9. 20°—4 days . . . . .	64.95 ± 0.086	2.045 ± 0.018	10.922 ± 0.055	13.542 ± 0.040
10. Untreated . . . . .	68.40 ± 0.014	1.018 ± 0.003	3.289 ± 0.012	4.481 ± 0.009
11. 30°—1 day . . . . .	66.15 ± 0.029	2.001 ± 0.007	9.006 ± 0.012	11.482 ± 0.020
12. 30°—2 days . . . . .	65.42 ± 0.062	2.379 ± 0.014	12.093 ± 0.035	15.109 ± 0.022
13. 30°—3 days . . . . .	62.56 ± 0.038	2.453 ± 0.025	14.114 ± 0.021	17.311 ± 0.048
14. 30°—4 days . . . . .	58.27 ± 0.048	3.498 ± 0.010	17.341 ± 0.080	21.752 ± 0.074
15. Untreated . . . . .	69.69 ± 0.052	0.885 ± 0.008	3.321 ± 0.016	4.381 ± 0.008
16. 40°—1 day . . . . .	61.97 ± 0.110	3.695 ± 0.037	14.930 ± 0.002	19.411 ± 0.034
17. 40°—2 days . . . . .	57.35 ± 0.071	5.413 ± 0.000	19.017 ± 0.023	25.431 ± 0.023
18. 40°—3 days . . . . .	51.43 ± 0.534	7.185 ± 0.049	20.892 ± 0.032	29.177 ± 0.015
19. 40°—4 days . . . . .	39.57 ± 0.024	9.410 ± 0.045	19.273 ± 0.057	29.698 ± 0.015

TABLE XVII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES GIVEN GLUCOSE IN EXPERIMENT 4 A, EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	0.495 ± 0.036	0.365 ± 0.029
2. 6°—1 day . . . . .	0.482 ± 0.030	2.235 ± 0.062
3. 6°—2 days . . . . .	0.326 ± 0.014	4.789 ± 0.003
4. 6°—3 days . . . . .	0.504 ± 0.004	6.111 ± 0.002
5. 6°—4 days . . . . .	0.575 ± 0.001	7.417 ± 0.034
6. 20°—1 day . . . . .	0.548 ± 0.042	1.086 ± 0.047
7. 20°—2 days . . . . .	0.997 ± 0.025	0.604 ± 0.007
8. 20°—3 days . . . . .	0.641 ± 0.004	1.018 ± 0.020
9. 20°—4 days . . . . .	0.931 ± 0.008	1.114 ± 0.026
10. Untreated . . . . .	0.512 ± 0.021	0.506 ± 0.018
11. 30°—1 day . . . . .	1.233 ± 0.081	0.768 ± 0.088
12. 30°—2 days . . . . .	1.218 ± 0.024	1.161 ± 0.009
13. 30°—3 days . . . . .	1.526 ± 0.039	0.927 ± 0.065
14. 30°—4 days . . . . .	2.088 ± 0.003	1.410 ± 0.014
15. Untreated . . . . .	0.782 ± 0.014	0.102 ± 0.022
16. 40°—1 day . . . . .	1.149 ± 0.019	2.546 ± 0.018
17. 40°—2 days . . . . .	1.889 ± 0.002	3.524 ± 0.001
18. 40°—3 days . . . . .	2.321 ± 0.021	4.864 ± 0.070
19. 40°—4 days . . . . .	3.320 ± 0.024	6.090 ± 0.021

Because of the interest and importance of these results, the experiment was repeated. In Experiment 4 B, blades were given 5 per cent glucose for three days at the following temperatures: 6°, 19.5°, 29.8°, and 39.9° C. The flasks in which the blades were placed were covered with wax paper, the blades being inserted

through slits. The residual solution was measured each morning, from which was calculated the amount of solution absorbed by four blades in one jar supplied with 400 cc. solution: the results of these measurements are presented in Table XVIII. The amount of solution absorbed was positively correlated with temperature each day. The amount of solution absorbed at a given temperature was negatively correlated with time (with one exception).

TABLE XVIII

EFFECT OF TEMPERATURE UPON ABSORPTION OF SOLUTION, EXPRESSED AS CC. ABSORBED BY 4 BLADES IN 1 JAR, IN EXPERIMENT 4 B

Temperature	1st day	2nd day	3rd day
6° C. ....	21	18	16
19.5° C. ....	22	21	20
29.8° C. ....	49	37	53
39.9° C. ....	120	104	82

TABLE XIX

MOISTURE AND SUGAR PERCENTAGES IN BLADES GIVEN GLUCOSE IN EXPERIMENT 4 B; MOISTURE DETERMINATIONS EXPRESSED UPON THE WET-WEIGHT BASIS, SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated ....	74.73 ± 0.057	1.123 ± 0.001	3.917 ± 0.017	5.246 ± 0.017
2. 6°—1 day ....	73.78 ± 0.181	3.351 ± 0.005	4.973 ± 0.003	8.587 ± 0.002
3. 6°—2 days ....	71.65 ± 0.024	5.498 ± 0.002	4.335 ± 0.006	10.062 ± 0.004
4. 6°—3 days ....	72.21 ± 0.105	6.882 ± 0.029	5.353 ± 0.078	12.517 ± 0.111
5. 19.5°—1 day ....	73.34 ± 0.114	1.914 ± 0.026	6.370 ± 0.008	8.619 ± 0.035
6. 19.5°—2 days ....	72.52 ± 0.081	2.198 ± 0.000	7.807 ± 0.002	10.417 ± 0.002
7. 19.5°—3 days ....	72.59 ± 0.095	2.595 ± 0.002	9.765 ± 0.041	12.375 ± 0.046
8. 29.8°—1 day ....	73.00 ± 0.038	2.111 ± 0.009	8.588 ± 0.025	11.152 ± 0.036
9. 29.8°—2 days ....	70.21 ± 0.000	2.531 ± 0.036	11.501 ± 0.065	14.618 ± 0.095
10. 29.8°—3 days ....	66.30 ± 0.076	3.752 ± 0.036	14.103 ± 0.070	18.597 ± 0.110
11. Untreated ....	74.31 ± 0.052	0.879 ± 0.008	4.528 ± 0.009	5.646 ± 0.001
12. 39.9°—1 day ....	68.33 ± 0.305	3.918 ± 0.039	16.352 ± 0.029	21.130 ± 0.070
13. 39.9°—2 days ....	61.45 ± 0.014	6.767 ± 0.039	20.365 ± 0.050	28.204 ± 0.013
14. 39.9°—3 days ....	50.91 ± 0.677	10.056 ± 0.083	19.864 ± 0.110	30.966 ± 0.199

The results of the determinations of moisture and sugars of Experiment 4 B are presented in Tables XIX and XX and Fig. 7. The results of Experiment 4 B (Fig. 7) closely resemble those of Experiment 4 A (Fig. 6) in showing an accumulation of glucose, but little formation of cane sugar at the lowest temperature, and increasing formation of sucrose with increasing temperature.

In Experiment 4 C, blades supplied with 5 per cent fructose were kept for three days at the following temperatures: 7°, 19.6°, 30.4°, and 39° C. The flasks were washed daily, the blades being trimmed and washed, and the fructose renewed at the same time.

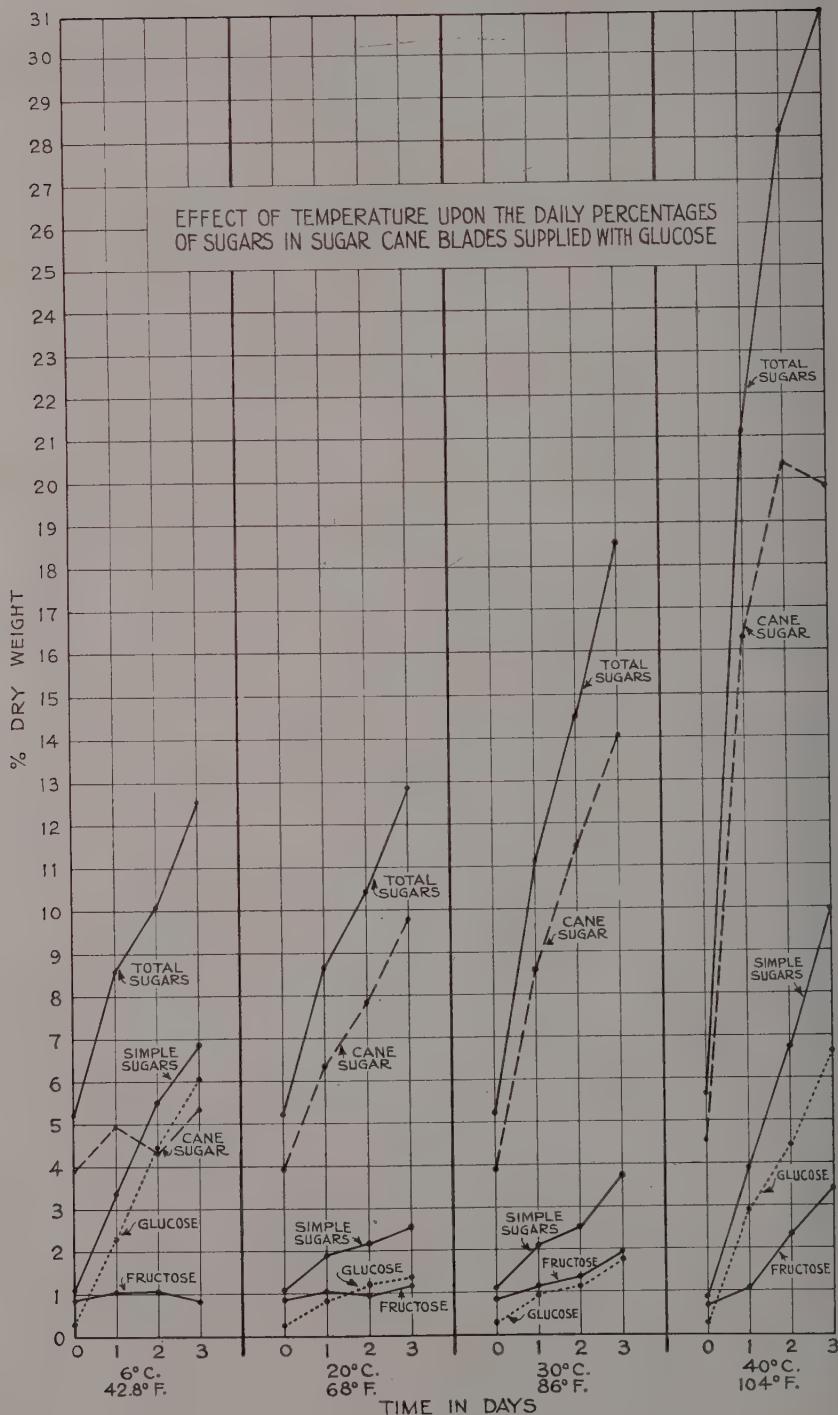


Fig. 7

TABLE XX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES GIVEN GLUCOSE IN EXPERIMENT 4 B, EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	0.836 ± 0.005	0.286 ± 0.004
2. 6°—1 day . . . . .	1.066 ± 0.070	2.285 ± 0.065
3. 6°—2 days . . . . .	1.079 ± 0.003	4.419 ± 0.006
4. 6°—3 days . . . . .	0.816 ± 0.010	6.066 ± 0.040
5. 19.5°—1 day . . . . .	1.059 ± 0.047	0.854 ± 0.021
6. 19.5°—2 days . . . . .	0.988 ± 0.039	1.210 ± 0.039
7. 19.5°—3 days . . . . .	1.203 ± 0.047	1.392 ± 0.050
8. 29.8°—1 day . . . . .	1.158 ± 0.034	0.953 ± 0.043
9. 29.8°—2 days . . . . .	1.372 ± 0.042	1.138 ± 0.069
10. 29.8°—3 days . . . . .	1.978 ± 0.104	1.773 ± 0.068
11. Untreated . . . . .	0.687 ± 0.014	0.192 ± 0.023
12. 39.9°—1 day . . . . .	1.064 ± 0.008	2.853 ± 0.048
13. 39.9°—2 days . . . . .	2.310 ± 0.082	4.457 ± 0.042
14. 39.9°—3 days . . . . .	3.409 ± 0.038	6.647 ± 0.045

TABLE XXI

MOISTURE AND SUGAR PERCENTAGES IN BLADES GIVEN FRUCTOSE IN EXPERIMENT 4 C; MOISTURE PERCENTAGES EXPRESSED UPON THE WET-WEIGHT BASIS, SUGAR PERCENTAGES UPON THE DRY-WEIGHT BASIS

Series	Moisture	Simple sugars	Cane sugar	Total sugars
1. Untreated . . . . .	74.33 ± 0.205	2.026 ± 0.005	3.819 ± 0.026	6.046 ± 0.022
2. 6°—1 day . . . . .	72.05 ± 0.100	4.369 ± 0.024	4.109 ± 0.028	8.695 ± 0.006
3. 6°—2 days . . . . .	71.94 ± 0.009	4.814 ± 0.002	4.098 ± 0.029	9.097 ± 0.018
4. 6°—3 days . . . . .	71.11 ± 0.086	6.210 ± 0.032	5.265 ± 0.004	11.752 ± 0.036
5. 19.6°—1 day . . . . .	73.00 ± 0.048	2.210 ± 0.022	4.988 ± 0.007	7.460 ± 0.029
6. 19.6°—2 days . . . . .	72.22 ± 0.048	2.379 ± 0.015	6.274 ± 0.048	8.983 ± 0.036
7. 19.6°—3 days . . . . .	71.85 ± 0.043	2.756 ± 0.026	7.633 ± 0.006	10.791 ± 0.020
8. 30.4°—1 day . . . . .	71.47 ± 0.019	2.408 ± 0.000	8.586 ± 0.031	11.446 ± 0.033
9. 30.4°—2 days . . . . .	66.56 ± 0.029	3.296 ± 0.007	9.715 ± 0.015	13.523 ± 0.008
10. 30.4°—3 days . . . . .	63.46 ± 0.057	3.777 ± 0.002	10.489 ± 0.003	14.818 ± 0.005
11. Untreated . . . . .	73.51 ± 0.024	1.621 ± 0.009	2.906 ± 0.007	4.680 ± 0.001
12. 39°—1 day . . . . .	69.84 ± 0.024	2.669 ± 0.000	10.943 ± 0.010	14.188 ± 0.011
13. 39°—2 days . . . . .	63.17 ± 0.000	4.207 ± 0.007	11.759 ± 0.010	16.585 ± 0.017
14. 39°—3 days . . . . .	52.14 ± 0.143	4.866 ± 0.008	11.691 ± 0.029	17.173 ± 0.023

TABLE XXII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES GIVEN FRUCTOSE IN EXPERIMENT 4 C, EXPRESSED UPON THE DRY-WEIGHT BASIS

Series	Fructose	Glucose
1. Untreated . . . . .	0.587 ± 0.029	1.439 ± 0.025
2. 6°—1 day . . . . .	3.144 ± 0.021	1.225 ± 0.003
3. 6°—2 days . . . . .	3.955 ± 0.001	0.859 ± 0.000
4. 6°—3 days . . . . .	5.562 ± 0.022	0.647 ± 0.054
5. 19.6°—1 day . . . . .	1.271 ± 0.040	0.938 ± 0.062
6. 19.6°—2 days . . . . .	1.809 ± 0.001	0.570 ± 0.013
7. 19.6°—3 days . . . . .	2.308 ± 0.014	0.447 ± 0.040
8. 30.4°—1 day . . . . .	1.933 ± 0.030	0.475 ± 0.029
9. 30.4°—2 days . . . . .	2.120 ± 0.010	1.175 ± 0.018
10. 30.4°—3 days . . . . .	2.424 ± 0.002	1.352 ± 0.000
11. Untreated . . . . .	0.949 ± 0.020	0.672 ± 0.011
12. 39°—1 day . . . . .	1.981 ± 0.032	0.687 ± 0.032
13. 39°—2 days . . . . .	2.873 ± 0.027	1.333 ± 0.034
14. 39°—3 days . . . . .	2.960 ± 0.006	1.906 ± 0.014

The results of the determinations of moisture and sugars are recorded in Tables XXI and XXII and Fig. 8. At 7° C., fructose was absorbed readily, but

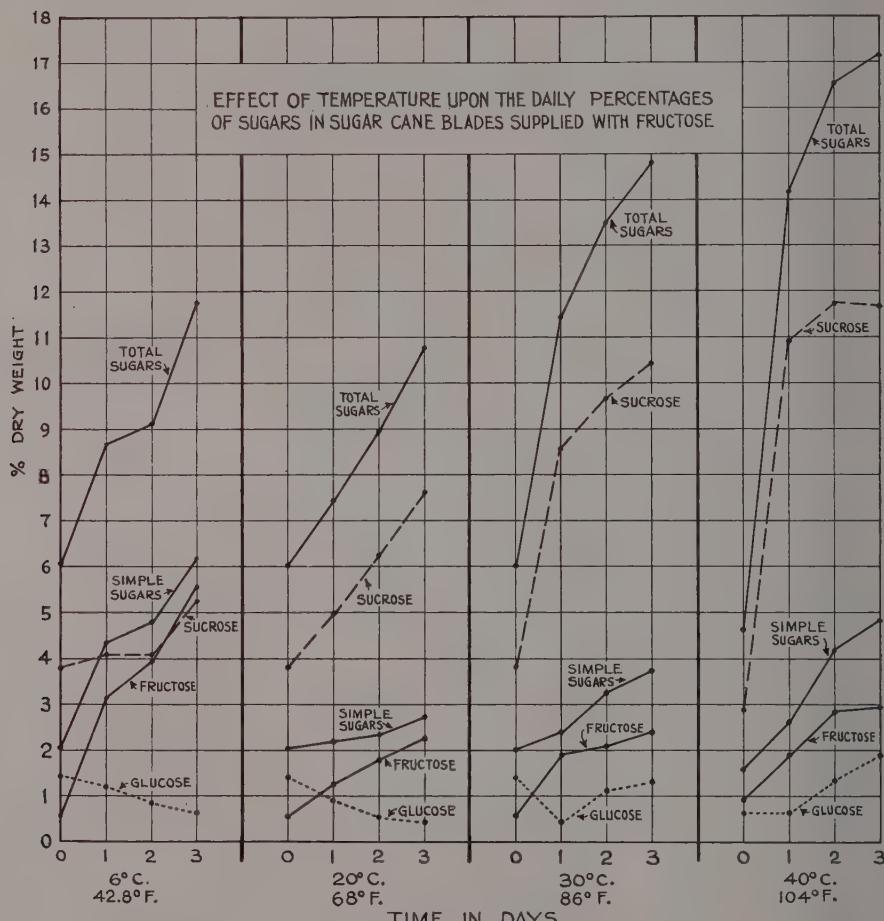


Fig. 8

it accumulated in the blades because very little was converted to glucose or sucrose. In fact, the conversion of fructose to glucose was so poor that the percentage of glucose decreased. There was no significant formation of cane sugar until the percentage of fructose equalled the percentage of sucrose.

At 19.6° C., the absorption of fructose was not quite as rapid as at 7° C. Sucrose increased considerably each day. Less fructose accumulated than at 7° C. The conversion of fructose to glucose was slow, as the percentage of glucose decreased each day.

At 30.4° C., the absorption of fructose was greater than at the lower temperatures, especially the first day. The percentages of sucrose increased con-

siderably the first day, after which time the increase in sucrose was smaller. Glucose decreased the first day and then increased.

At 39° C., the absorption of fructose was greater than at 30.4° C. More sucrose was formed from fructose at 39° than at 30.4° C. the first day, but not the second day, and the third day there was a slight loss in sucrose. There was an accumulation of both glucose and fructose.

Experiment 4 C, in which the blades were supplied with fructose, resembles Experiments 4 A and 4 B, in which the blades were supplied with glucose, in showing similar trends, but the magnitudes are all smaller in Experiment 4 C. Considerably less fructose than glucose was absorbed at 40° C. When supplied with glucose at 40° C. the total sugars reached more than 30 per cent on the third day, but when supplied with fructose the total sugars reached only 17 per cent.

Experiments 4 A, 4 B, and 4 C show that at 7° C. whether supplied with glucose or fructose, the blades increase in the simple sugar supplied and not in the other simple sugar. Therefore low temperature inhibits the interconversion of glucose and fructose. Whichever sugar was supplied, its absorption was as great at 7° C. as at 19° C. Both the interconversion of glucose and fructose and the formation of sucrose increased with temperature.

The large increase in sucrose in Experiments 4 A, 4 B, and 4 C is the result not only of time and temperature but also of concentration. Table XVIII shows that the amount of solution absorbed increased with temperature. Since it is probable that the sugar solution enters the cut blade by mass flow, the blades held at the higher temperatures received more simple sugar than the blades held at the lower temperatures. To determine whether or not temperature has an effect upon the synthesis of sucrose not complicated by the effect of temperature upon absorption, the synthetic efficiencies of the blades in these experiments were calculated and are plotted in Fig. 9. As already explained, the synthetic efficiency is the percentage of sugar absorbed that is converted into sucrose. Fig. 9 shows that only a small percentage of the sugar absorbed at 6°–7° C. was converted to sucrose. The greatest difference in synthetic efficiency lay between 6°–7° C. and 20° C. In all three experiments the synthetic efficiency for the first day was greater at 30° than at 20° or 40° C., but this relationship did not always hold on the following days. The synthetic efficiency decreased with time at 40° C. It is felt that the synthetic efficiency for the first day is the truest measure of the effect of temperature, inasmuch as it is well known that the rate of reactions is decreased by the accumulation of end products. The results of these experiments agree with Experiment 3 (Table XV) in showing that the optimum temperature for the synthesis of sucrose is approximately 30° C.

#### DISCUSSION

The experiments on the formation of cane sugar by sugar cane blades supplied with simple sugar during varying lengths of time, as summarized in Fig. 4, show that the blade of the sugar cane plant has a remarkable ability to make and accumulate cane sugar. The normal leaf attached to a stalk never attains as high a sugar content as that found in these detached blades, because the sugar is translocated from the blade to the stem. Even when the blades became yellow and dried at the tips, the accumulation of sucrose continued. This does not mean

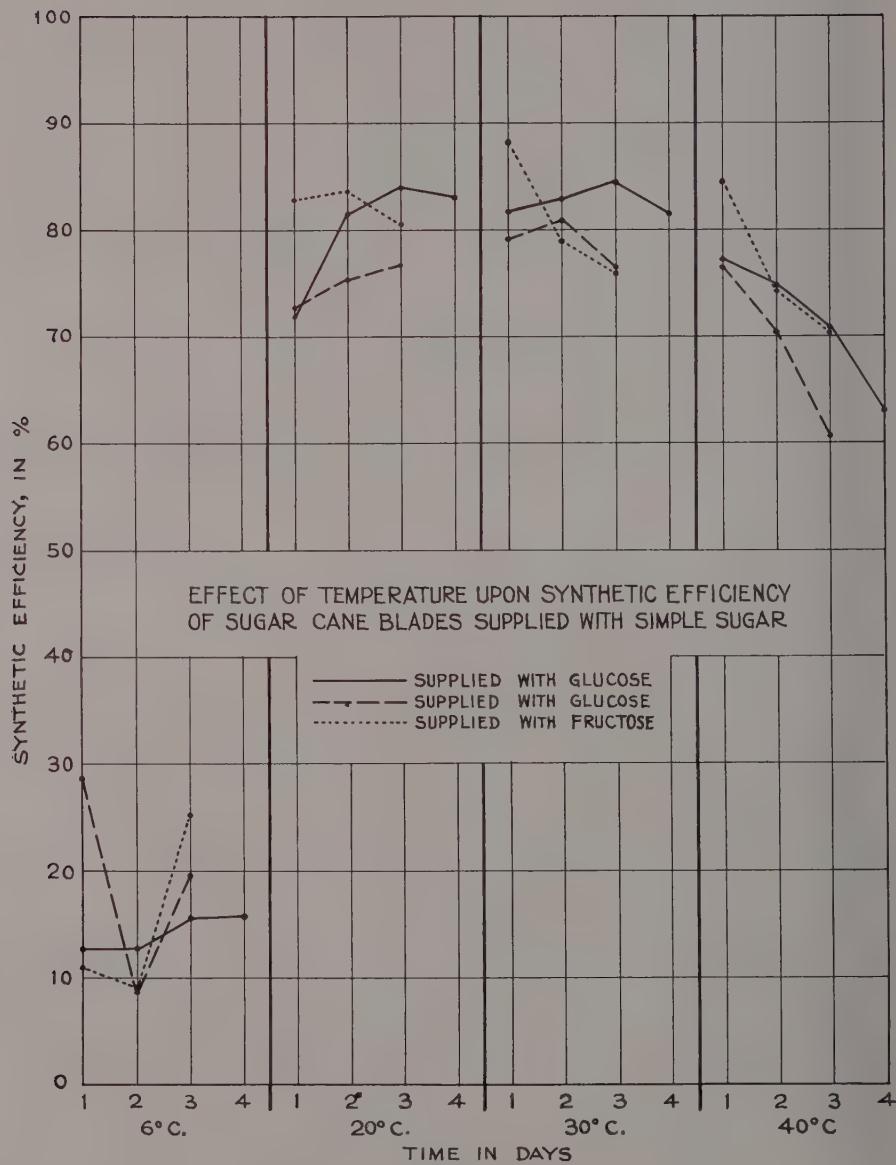


Fig. 9

that yellowed blades make sucrose in the field, because for the manufacture of sucrose raw materials are needed; in the field they are supplied in the process of photosynthesis (which requires chlorophyll) whereas in these experiments the raw materials were solutions of glucose and fructose in which the cut ends of the blades were placed. The curve for cane sugar in Fig. 4 shows that the amount of sucrose made per day decreased with time. This was due both to the poor

condition of the blades toward the end of the experiment and to the accumulation of sucrose itself, since the accumulation of end products is known to decrease the speed of chemical reactions.

The effect of temperature was studied not only to gain an insight into the inter-conversions of the sugars but also to determine the optimum temperature for the formation of sucrose. Table XV shows that 93 per cent of the sugar absorbed at 30° C. was converted into sucrose, compared with 76 per cent at 19° and 52 per cent at 40° C. As discussed under Experiment 4, those results also indicate that the optimum temperature for the manufacture of sucrose is approximately 30° C. (86° F.). Leonard (4) working with corn blades supplied with 6 per cent glucose at 8°, 30°, 40°, and 50° C. found the greatest synthetic efficiency at 40° C.

The question arises as to why synthesis was less efficient at 40° than at 30° C. It is obvious that the mechanism of synthesis was not destroyed, because of the large accumulation of sucrose. Evidently heat increased the absorption of simple sugar more than it increased the formation of sucrose. These results illustrate the difficulty of differentiating between the effect of temperature upon the absorption of simple sugar and upon the formation of cane sugar. The curves for total sugars in Figs. 6, 7, and 8 show that the absorption of simple sugar increased with temperature from approximately 10° C. to 40° C. The formation of sucrose, however, was best at 30° C., as already mentioned. Since absorption took place readily but little or no sucrose was formed at 10° C., it was thought that blades should be allowed to absorb at the low temperature and then be transferred to higher temperatures for synthesis. So far the experiments carried on in this manner have given no evidence of synthesis, probably because the blades were allowed to absorb for only one day, which was not long enough to raise the simple sugar percentage above the percentage of cane sugar. If blades are allowed to absorb glucose for four days at 10° C., the percentage of glucose will become almost double the percentage of sucrose. Such blades placed at higher temperatures should synthesize sucrose. This should provide a method of distinguishing between the effect of temperature upon absorption and its effect upon synthesis. This experiment will be conducted soon and the results reported later.

The results reported in this investigation provide decided evidence that the simple sugars, glucose and fructose, unite to form cane sugar. These experiments with detached blades supplied with simple sugar in the dark, show that at moderate temperatures (20°–30° C.) the fluctuations in percentages of glucose and fructose are only slight, whereas at extreme temperatures, either very low (6°) or very high (40°–50° C.) the percentages of simple sugars increase greatly. At the low temperature the percentage of simple sugar increases because the particular simple sugar absorbed is not converted to the other simple sugar or to sucrose; at the high temperatures the percentage of simple sugar increases because the absorption of simple sugar is increased more than the formation of sucrose. The slight fluctuations in simple sugars at moderate temperatures in these experiments bear a strong resemblance to the slight fluctuations in simple sugars in attached leaves carrying on photosynthesis (1). Thus the evidence is becoming even stronger that in photosynthesis as well as synthesis in the dark the simple sugars, glucose and fructose, are only fleeting intermediates and that cane sugar is a temporary storage product in the blade of the sugar cane plant. However, emphasis must be laid on the fact

that these experiments do not prove conclusively that the simple sugars are made before sucrose in photosynthesis. They prove that sucrose can be made from the simple sugars, glucose and fructose, but they do not prove that that is the only way or even the chief way in which sucrose is made.

These experiments indicate that glucose and fructose are interchangeable in the leaf, that sucrose is made from glucose and fructose, that the rate of these processes decreases with time because of the piling up of the end product, and that these processes increase with temperature reaching a maximum at 30° C. The fact that the optimum temperature for the formation of sucrose is 30° C. may indicate that the mechanism of synthesis is an enzyme with optimum activity at that temperature, or that the colloidal condition of the protoplasm is more favorable for synthesis at that temperature, or some other factor may be involved.

Interest naturally centers upon the mechanism of synthesis. To obtain some slight insight into the mechanism of synthesis, the temperature coefficients for absorption and synthesis were calculated. The temperature coefficient is the ratio of the rate of a process at one temperature to the rate of the process at a temperature 10° C. lower, and is expressed by the symbol,  $Q_{10}$ . Both chemical reactions and physical processes are affected by temperature, but in different degrees. The temperature coefficient for most chemical reactions at ordinary temperatures (10°–40° C.) is generally 2–3, being generally a little more at the lower range of temperature and a little less at the higher range of temperature. Expressed in another way, the rate of most chemical reactions is doubled or trebled by a rise in temperature of 10° C. But the temperature coefficient for most physical processes is generally close to one. Thus the temperature coefficient may be used to determine whether a complex phenomenon is limited by a physical process or by a chemical reaction. In this study temperature coefficients have been calculated both for absorption and for synthesis.

The temperature coefficients for absorption were calculated in two ways: first, the amount of solution absorbed as shown by the amount left, and second, the increase in total sugars in the blades. The first method gives the absorption of solution, presumably water plus glucose, while the second method gives the absorption of glucose alone. In the second method no account is taken of the glucose that is absorbed and used in respiration, the synthesis of proteins, or any processes other than the synthesis of sucrose, but it is thought that the error due to these necessary omissions is relatively slight.

The temperature coefficients for the absorption of solution in Experiment 4 B were calculated from the data presented in Table XVIII, the results for the three days were averaged, and are recorded in Table XXIII.

TABLE XXIII

TEMPERATURE COEFFICIENTS FOR ABSORPTION OF SOLUTION, ABSORPTION OF SIMPLE SUGAR, AND SYNTHESIS OF SUCROSE, CALCULATED FROM EXPERIMENT 4, EXPRESSED AS  $Q_{10}$

Temperature range	Absorption of solution	Absorption of simple sugar	Synthesis of sucrose
10–20° C. ....	0.82	0.93	3.72
20–30° C. ....	2.00	1.90	1.09
30–40° C. ....	2.26	1.21	0.95

Calculations indicate that absorption of solution at a low temperature was a purely physical process. At higher temperatures, however, some chemical reaction or reactions aided the absorption of solution.

The temperature coefficients for the absorption of simple sugar, as measured by the increase in total sugars in Experiments 4 A, 4 B, and 4 C, were calculated from the data recorded in Tables XVI, XIX, and XXI, and were then averaged and presented in Table XXIII. These coefficients indicate that the absorption of simple sugar was purely physical at 10°–20° C., but chemical at 20°–30° C.

When the coefficients for absorption of solution are compared with the coefficients for absorption of simple sugar, both processes appear to be limited by physical means at the lower temperatures, but by chemical means at the higher temperatures.

One interesting point brought out by these coefficients is that under certain conditions the absorption of solution and the absorption of simple sugar may be limited by chemical means. If absorption depends upon a chemical reaction, then it would seem that the solution of simple sugar cannot enter and move up a cut blade merely by mass flow.

The temperature coefficients for the synthesis of sucrose were calculated from the synthetic efficiencies using the results of Experiments 4 A, 4 B, and 4 C. Because the synthetic efficiencies for the first day seemed the most reliable as mentioned under "Results," the coefficients for the first day only are presented. The coefficients for the three experiments were averaged and are recorded in Table XXIII, which shows that synthesis was limited by a chemical reaction at the lower temperature, which is only to be expected. What is more difficult to explain is the fact that at 20°–40° C. the temperature coefficients were those of a physical process.

To sum up, the temperature coefficients obtained in this investigation suggest that absorption at low temperature was a purely physical process, but that at higher temperatures absorption was limited by a chemical reaction. The synthesis of sucrose, however, was limited by a chemical reaction at lower temperatures, but by a physical process at higher temperatures. Just what chemical and physical principles are involved is not apparent from this study.

#### SUMMARY

- Experiments are described dealing with time and the effect of temperature upon the interconversion of glucose and fructose and formation of cane sugar by detached blades of the sugar cane plant supplied with simple sugar in the dark.

- Supplying the blades with both glucose and fructose hastens and increases the formation of cane sugar in the dark, compared with supplying only one of the simple sugars.

- Detached blades of the sugar cane plant can continue to form sucrose from glucose for nearly two weeks, reaching over 16 per cent cane sugar on the dry-weight basis.

- Temperature affects the absorption of simple sugar, the interconversion of glucose and fructose, and the formation of sucrose.

5. The absorption of simple sugar is much the same at 6° and 20° C., but is increased considerably from 20°–40° C.

6. The interconversion of glucose and fructose does not take place at 6° C.; it keeps pace with the formation of sucrose at 20° C.; and at 30°–40° C. it is increased.

7. The optimum temperature for the formation of sucrose was found to be 30° C.

8. The temperature coefficients obtained in this investigation suggest that absorption at low temperature (6°–20° C.) was a purely physical process, but at higher temperatures (20°–40° C.) absorption was limited by a chemical reaction. The synthesis of sucrose, however, was limited by a chemical reaction at lower temperatures but by a physical process at higher temperatures.

9. The evidence indicates that the simple sugars are fleeting intermediates in the leaf and that cane sugar is a storage product.

#### ACKNOWLEDGMENTS

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## Factors Affecting the Germination of Sugar Cane\*

By HARRY F. CLEMENTS†

### INTRODUCTION

Although the mechanics of planting sugar cane is a comparatively simple process involving cutting the cane stem into short pieces, dropping these into a furrow and covering them with soil, the transition within the cane from an upright, growing plant to a storage organ from which several buds develop is an abrupt one involving many, deeply seated changes. Before it is cut, the stem acts as a mechanical support of the leaves, conducts water and minerals upward while at the same time it conducts sugars and perhaps other materials downward. Some of these it stores within its own tissues; some it uses in its own respiration; and some it moves on to the roots. Furthermore, though the bud which is found at each node is a complete growing point, it is dormant not because of any incapacity to grow but because of certain substances produced in the upper parts, which move downward and inhibit the processes which normally would make for growth.

After the seed piece is laid in the soil and covered, all of these processes change. Now the bud inhibitors are no longer moving into the dormant buds and, after a day or two, the last traces of them disappear. The bud becomes active. Also, the root primordia, which were lying dormant in the root band, become active. The respiration of these structures increases enormously. Foods which were stored in the tissues of the cane stem are now mobilized and moved to the buds to provide the energy required to build new tissues, help force the young roots into the soil and lift the young shoot out of the soil. Enzymes are active during these stages. Shortly after the shoot emerges, it develops its own root system and finally becomes independent of the old seed piece.

But while this is going on, soil organisms are also active. The seed piece with its cut ends and its large amount of sugar is an excellent medium for their growth. They begin to invade the cane and, if conditions are relatively more favorable for their development than for that of the buds, the seed piece rots and the buds fail to emerge. If conditions are more favorable to the development of the bud, the young shoot emerges and becomes independent before the soil organisms take possession of the cane.

Germination, then, is something of a race between the growth processes of the buds and roots and the destructive effects of the soil organisms. Fortunately, where all or most factors affecting the growth of the buds are favorable, vigorous, rapidly growing shoots are produced. To determine the optimum conditions under which the various factors operate in germination is the object of this work.

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### EXPERIMENTAL

As a result of these studies, the factors affecting the germination are grouped into two general categories: factors external to the cane; and factors internal to the cane. The most important external factors are soil temperature, soil moisture, and soil oxygen. The important internal factors are the age of the seed pieces, their length with particular reference to the number of nodes, the position of the buds on the seed pieces, their composition and the presence or absence of the leaf sheaths.

*General Procedure:*

In working out the effects of these various factors on germination, variety H 109 was used throughout. Cane which was about ten months old was cut into three pieces: the top third, the middle third and the bottom third. The seed pieces from the top third are referred to in the tables as No. I, the middle third as No. II, and the bottom third as No. III.

Except as otherwise specified, the pieces were cut into 3-bud lengths. The buds were carefully inspected and defective ones discarded, so that where a given number of buds was used, it is understood that they were all apparently capable of growth.

The seed pieces were dipped into a Ceresan solution before planting in soil flats. Unless otherwise specified, the pieces were placed so that the buds were on the side. The flats were placed in the open, and watered as needed. As soon as the shoots began to emerge, daily counts were taken. From these data, the average time of emergence was obtained. After about one month, the seed pieces were removed from the soil and washed. The length of the shoots from the point of attachment on the old cane seed piece to the tip of the longest leaf was determined. In several series, the roots which developed from the seed piece and also those coming from the young shoot were counted. In this way, a complete record was available for each node on each seed piece, including roots and shoot. These studies involved the use of 75,000–100,000 buds.

### EXTERNAL FACTORS

*Soil Temperature:*

Preliminary tests showed that the temperature of the soil was perhaps the most important single factor in germination. Although equipment for temperature controls was not available, markedly different temperatures were obtained by planting one set of flats in direct sunlight, another in a well-ventilated greenhouse, and a third in the heavy shade of some trees. In one series (Table I), the temperature of the soil in the flats in the open, exposed to direct sunlight, ranged from 75° upward to an average daily maximum of 91.8° F., that in the greenhouse from 75° F. to an average daily maximum of 88° F., and that in the deep shade from 69° F. to 73.4° F.

The highest temperature compares with very favorable field conditions, while the low temperature is about the average condition found in cloudy areas during the winter months.

Twenty-five three-bud seed pieces were selected from Nos. I, II, III pieces, respectively, and planted October 15, 1938. These plantings were taken up November 18 and measured. The data are reported in Table I.

TABLE I  
EFFECT OF SOIL TEMPERATURE ON GERMINATION

Age of seed pieces*	Soil temperature		Per cent emergence†	Average emergence time (days)
	average daily maximum (° F.)			
I	91.8		93.3	10.3
	88.0		88.9	12.7
	73.4		50.5	20.4
II	91.8		74.4	13.5
	88.0		64.7	13.8
	73.4		31.8	21.9
III	91.8		77.7	13.8
	88.0		66.6	14.2
	73.4		45.2	24.2

\* No. I pieces are taken from the top green-leaf section, No. II pieces from the middle third of the cane, and No. III pieces are from the bottom third of the cane.

† Although most all of the buds planted actually start growth, not all of them emerge from the soil. Only those which actually emerge are counted.

Another series was planted June 20, 1939, and harvested August 17, 1939. The average daily maximum temperature in the exposed place averaged 89.0° F., in the shade 71.0° F. The results of this test are shown in Table II.

TABLE II  
EFFECT OF SOIL TEMPERATURE ON GERMINATION

Age of seed pieces	Soil temperature		Per cent emergence
	average daily maximum (° F.)		
I	89.0		85.3
	71.0		58.6
III	89.0		65.3
	71.0		26.7

The results of still another series, planted October 12, 1939, and harvested December 8, 1939, are reported in Table III. The soil in which this series was planted differed from the above in that it was very heavy.

TABLE III  
EFFECT OF SOIL TEMPERATURE ON GERMINATION

Age of seed pieces	Soil temperature		Per cent emergence	Average emergence time (days)
	average daily maximum (° F.)			
I	90.0		88.0	15.6
	71.0		36.0	36.8
II	90.0		73.3	17.8
	71.0		26.6	40.1
III	90.0		54.7	20.4
	71.0		17.3	48.9

It is clear from these data that soil temperatures exert a tremendous influence on the germination processes. This, to be sure, is to be expected, but it is somewhat surprising that temperatures at or slightly above 70° F. are so nearly marginal for sugar cane growth. Germination at the low temperature is roughly one half what it is at the higher temperature and the emergence time is nearly doubled.

In an effort to determine the importance of this factor with respect to germination under field conditions, soil temperatures of bare fields\* at Waipio and also on the Kailua uplands (cloudy) were measured. Six matched thermometers were inserted in the soil at the 1-, 2-, 3-, 4-, 5- and 6-inch depths, respectively. The temperature was read at intervals during the day. In Table IV are reported the records gathered at Kailua on December 27, 1938. On this day the sky was heavily overcast and a light rain fell early in the day. By noon, the rain had ceased, and the sun shone intermittently until after 2:30 p.m. On April 1, 1939, another series of readings was made. This day was quiet and fairly clear. These records are reported in Table V. On June 30, 1939, the day was cloudy. The soil temperatures are shown in Table VI. Similar records appear in Tables VII, VIII and IX for Waipio.

TABLE IV  
SOIL TEMPERATURE AT KAILUA (° F.)  
DECEMBER 27, 1938—CLOUDY

Time	Depth of thermometer bulb—					
	1"	2"	3"	4"	5"	6"
10:00 a.m.	69.8	68.0	67.1	67.1	67.1	66.2
12:45 p.m.	77.2	69.8	68.9	68.0	68.9	68.0
2:30 p.m.	77.2	71.6	70.7	68.9	69.8	68.0

TABLE V  
SOIL TEMPERATURE AT KAILUA (° F.)  
APRIL 1, 1939—CLEAR

Time	Depth of thermometer bulb—				
	1"	2"	3"	4"	5"
10:30 a.m.	82.4	75.2	74.5	73.4	71.6
11:00 a.m.	88.7	77.9	77.0	73.4	72.5
12:30 p.m.	92.3	82.4	78.8	76.1	74.5
2:00 p.m.	85.1	82.4	80.6	77.9	76.1
3:00 p.m.	82.4	81.5	80.6	78.8	78.8
4:00 p.m.	80.6	80.6	79.7	77.0	77.0

TABLE VI  
SOIL TEMPERATURE AT KAILUA (° F.)

Time	Depth of thermometer bulb—					
	1"	2"	3"	4"	5"	6"
6:30 a.m.	73.4	73.4	73.4	75.2	75.2	76.1
7:30 a.m.	77.0	75.2	75.2	75.2	75.2	75.2
8:30 a.m.	77.9	76.1	76.1	75.2	75.2	76.1
9:30 a.m.	81.5	77.0	75.2	75.2	76.1	75.2
1:15 p.m.	91.4	80.6	78.8	77.0	77.0	76.1

\* It should be clear that temperatures of the top layer of soils which are without vegetation will be very different from those with plant cover.

TABLE VII

SOIL TEMPERATURE AT WAIPIO (° F.)  
DECEMBER 27, 1938—CLEAR

Time	Depth of thermometer bulb					
	1"	2"	3"	4"	5"	6"
8:00 a.m.	68.2	68.0	70.7	70.7	71.6	73.4
9:00 a.m.	69.8	68.0	70.7	70.7	72.5	73.6
10:00 a.m.	77.9	70.7	71.6	71.6	72.9	74.3
11:30 a.m.	86.9	78.8	76.1	73.4	73.4	74.3
1:00 p.m.	87.8	81.5	76.1	74.3	74.3	74.3
3:30 p.m.	84.2	82.4	76.1	75.2	75.2	75.2

TABLE VIII

SOIL TEMPERATURE AT WAIPIO (° F.)  
MARCH 30, 1939—VERY BRIGHT, QUIET DAY

Time	Depth of thermometer bulb				
	1"	2"	3"	4"	5"
8:00 a.m.	68.0	69.8	69.8	69.8	73.4
9:00 a.m.	77.0	75.2	73.4	71.6	74.3
9:45 a.m.	86.0	82.5	75.2	75.2	75.2
10:30 a.m.	89.6	87.8	77.0	77.0	75.2
11:30 a.m.	96.8	93.2	80.6	80.6	77.0
1:30 p.m.	112.1	95.0	82.4	84.2	79.7
3:00 p.m.	98.6	91.4	86.0	86.0	80.6

TABLE IX

SOIL TEMPERATURE AT WAIPIO (° F.)  
JUNE 29, 1939—SUN VERY BRIGHT, STRONG TRADE WIND

Time	Depth of thermometer bulb					
	1"	2"	3"	4"	5"	6"
8:30 a.m.	77.0	77.0	77.0	78.8	77.0	79.7
9:30 a.m.	81.5	80.6	77.0	79.7	77.9	79.7
10:30 a.m.	85.1	84.2	79.7	81.5	78.8	79.7
12:00 m.	89.6	86.0	84.2	84.2	82.4	80.6
12:45 p.m.	89.6	89.6	84.2	84.2	83.3	80.6
2:15 p.m.	90.5	89.6	85.1	84.2	83.3	82.4
3:30 p.m.	91.4	91.4	86.0	86.0	85.1	84.2

In view of the germination behavior at high and low temperatures, shown in Tables I to III, and the actual soil temperatures found under field conditions, it is quite clear that this is the most important single factor operating under field conditions.

At Kailua during the late fall and early winter months, the soil temperature is certainly marginal or sub-marginal for the germination of sugar cane. Table IV shows that for that particular day the temperature for the most part was below seventy. Clearly, had a planting been made at a time when the two or three weeks following were cloudy, the planting would have been wasted. The tendency for the upper inch of soil to warm when the sun shines intermittently emphasizes the desirability of shallow planting during unfavorable seasons.

On April 1, 1939, at Kailua (Table V), the day was clear except for the clouds

high overhead which gather about the mountain tops. The temperature in the upper five inches ranged from 71.6° to 92.3° F. Even this late in the season, it would still be unwise to plant cane at depths greater than three inches.

On June 30, 1939, the Kailua soil ranged in temperature from 73.4° to 91.4° F. Although this was summer, the cloudy weather made for cool soils.

In places where nights are cold and the days show a tendency toward cloudiness, it is obvious from these data that planting must be shallow. At times it would be advantageous to bury the butts of the pieces and allow the terminal ends to actually protrude from the soil to take advantage of the sun's warmth.

The soil temperature records at Waipio indicate that successful plantings should be much more easily obtained than at Kailua, but even there some attention must be paid to the idiosyncrasies of soil temperature.

On December 27, 1938, the day at Waipio was clear. Nights at that season were cold. The soil was very cold in the early morning hours (Table VII). Some residual heat remained at the 6-inch level. But above that, the soil was too cold for cane growth. However, from 10:00 a.m. on, the upper two inches of soil were warm enough to encourage growth. Even at Waipio, which would ordinarily be described as sunny, when winter plantings have to be made, it is necessary to make shallow plantings.

Spring temperatures at Waipio may be most favorable. In Table VIII, the soil temperatures on a clear day are recorded. The temperatures range from 68° to 112.1° F. Obviously, with such temperatures, shallow plantings will be completely successful. The cooling effect of the strong trade winds might be surmised, but in Table IX, the soil temperatures on a bright but windy day are recorded (June 29, 1939). The effect of the wind is to reduce the maximum temperature reached. In the summer, however, the soil even at the 6-inch level is warm enough to facilitate growth. At such times, even careless plantings may be completely successful.

Although the gathering of soil temperature data was discontinued, occasional measurements were made. At one time, in the middle of July, the temperature of the top two inches of semi-dry soil at Waipio reached the very high figure of 140° F. Under such conditions, in order to avoid the excessive heat and to obtain sufficient moisture, it would seem wise to plant more deeply than in winter or spring.

To summarize the data so far presented, it seems clear that temperatures slightly above 70° F. are marginal for germination of sugar cane. Such temperatures are not uncommon in Hawaii during the winter months and especially in cloudy areas. But, even in sunny areas, the soil temperatures during the winter must be taken into consideration. Where winter plantings are necessitated by schedules, shallow plantings will take advantage of the slight warmth which obtains.

During the summer months in sunny, warm places such as Waipio, planting may be done in haphazard fashion and, because of the very warm soil even to depths of six inches, successful stands will be obtained. But in cloudy areas, even in summer, attention must be focussed on the temperature of the soil.

A problem of considerable importance to the planter develops as a result of these considerations. Many fields are on mountain sides where cold nights and cloudy days are the rule. What can be done to aid germination under such

conditions? This whole problem commanded a good deal of attention during these studies and will be presented in detail at the end of this paper. A number of simple seed-piece treatments are available for such conditions.

*Soil Aeration and Soil Moisture:*

The two important external factors other than soil temperature are soil aeration and soil moisture and because of their interdependence are considered together. Since there is no precise way of maintaining certain levels of oxygen and moisture in the soil, no quantitative data are available. Observations made during the two and a half years of study, however, emphasize the importance of these two factors.

Where the soil is very porous, aeration is abundant, but under these conditions moisture is likely to be limiting. When the soil is low in moisture especially under cool conditions, root development is poor and in many cases no roots will develop at all. The buds will develop, but many of them die before producing their own roots.

On the other hand, where the soil is very heavy with a strong tendency to puddling, soil moisture is overly abundant but soil oxygen becomes the limiting factor. Under such conditions, even though the soil is warm, germination will be very poor. During some of the studies made, some flats became badly puddled and, even though the planting material was excellent and the soil warm, not a single bud emerged out of the twenty-five planted. In Table III are reported the results of germination in a test in which a heavy soil was used. Two points are of interest. First, germination of old body pieces is materially reduced even under favorable temperature conditions. Second, emergence time is considerably increased. Oftentimes in the field one sees similar conditions. In low spots in the irrigation furrow, where water stands and puddling develops, long stretches may develop with an insufficient germination or a delayed germination resulting in an unsatisfactory stand.

Under field conditions, where irrigation is practiced, soil aeration can in part be controlled through less frequent applications of water. Where soils are heavy and rainfall is heavy, the seed pieces may have to be planted either up on the side of the furrow or with the butt in the soil and the top protruding. After emergence, the soil may be worked around the seed piece.

The depth of planting is one of the best tools available for reconciling the three external factors with good germination. Shallow planting is necessary in cool, poorly aerated, and poorly drained soils. Deeper planting is desirable in well-drained soils especially during hot summer days. The depth of planting should vary from winter to summer, from wet to dry areas, from heavy soils to light soils. As is shown in Table IV, planting sugar cane even 2 inches below the surface would be unsatisfactory.

In January a quarter-acre planting was made at Kailua. The seed pieces were just barely covered with soil. In fact many of the pieces were partly exposed, but a perfect stand was obtained. On the other hand, in August another quarter-acre was planted. The days were clear and warm. The seed pieces were covered to a depth of about 4 inches. Again a perfect stand was obtained, but a deep

planting in November resulted in about a 25 per cent germination and a 50 per cent stand.

These, then, are the results of germination studies dealing with the external factors of soil temperatures, aeration and moisture. Any one of these factors, either its lack or excess, may cause complete failure in planting. Since they are so definitely interrelated, they should be considered together.

#### INTERNAL FACTORS

Internal factors affecting the germination of sugar cane are conveniently listed under the following headings: Age of seed pieces, their length, the position of the buds, the composition of the seed pieces, and the presence or absence of leaf sheaths (trash). These will now be considered in their order.

##### *The Age of the Seed Pieces:*

It is obvious to any one who has worked with cane that body pieces are less desirable for planting than top pieces. Old body pieces, in general, are less desirable than the younger body pieces. The reasons for this are at times fairly obvious. At other times, they are not. Generally speaking, the older a bud is the better is its chance of having been injured either by insects or through mechanical means. Considerable amounts of cane were examined in which every bud on the lower half of 10-11 months' old cane had been destroyed by some insect which had penetrated to the growing tip of each bud, and had tunneled through it to the base of the bud. The presence of the tunnel is usually indicated by a small opening in the tip of the bud.

Many buds on the body pieces are somewhat developed and are easily damaged through handling. But buds on old pieces which appear to be perfectly normal will produce slower growing shoots and a lower germination percentage than will young buds (Tables I, II, III). Also, the old buds are relatively much less successful under unfavorable soil temperature and aeration conditions. (See especially Tables II and III.) Furthermore, as will be seen later, the old buds do not respond well to stimulation treatments. For these various reasons, old body pieces should never be used except under the very best of planting conditions and when younger material is not available. One other recommendation might well be made at this point. In view of the marked difference in vigor of old and young buds, it seems clear that, where old as well as young buds must be used, they should be segregated before planting.

##### *Length of Seed Piece and Position of the Buds:*

These two internal factors affecting germination are so intimately associated with one another that they will be treated together.

To begin with, studies were conducted to determine whether all buds on 10-11 months' cane are capable of growth. In this study thirty canes were selected on which all of the buds were apparently healthy. The canes were cut into pieces, each carrying a single bud. These single bud pieces were planted in soil in the order they occurred on the cane.

The single bud pieces from ten canes were planted with the buds up, those from ten others with the buds down, and those from ten more with the buds on the side. The buds were numbered from the top toward the bottom. In other words, bud No. 1 was the youngest on the cane. The plantings were made April 13, 1939, under favorable temperature conditions. Emergence records were compiled. On May 27, 1939, the plants were taken up and measured. The results of this study are reported in Tables X, XI and XII.

TABLE X  
BEHAVIOR OF SINGLE BUD PIECES WHEN PLANTED WITH THE BUDS  
ON THE UPPER SIDE  
(Average of 10)

Bud No.	Length of shoot (cm.)	Time for emergence (days)
1 .....	37.5	9.0
2 .....	46.3	8.1
3 .....	51.5	6.9
4 .....	48.7	6.8
5 .....	35.7	6.7
6 .....	39.0	6.8
7 .....	35.0	7.4
8 .....	36.3	7.5
9 .....	35.5	8.5
10 .....	30.6	9.1
11 .....	29.1	9.7
12 .....	25.8	9.5
13 .....	25.9	11.0
14 .....	25.1	12.2
15 .....	25.1	13.0
16 .....	25.6	13.1
17 .....	25.1	11.8
18 .....	26.7	12.2
19 .....	27.6	12.1
20 .....	30.6	12.6
21 .....	26.9	12.9
22 .....	32.4	14.2
Average .....	32.45	10.5

TABLE XI  
BEHAVIOR OF SINGLE BUD PIECES WHEN PLANTED WITH THE BUDS  
ON THE UNDER SIDE  
(Average of 10) --

Bud No.	Length of shoot (cm.)	Time for emergence (days)
1	42.0	14.8
2	54.5	14.3
3	57.2	14.4
4	45.2	15.2
5	38.5	16.9
6	40.1	15.8
7	38.3	17.7
8	34.3	19.1
9	36.3	21.5
10	35.6	22.2
11	32.6	24.8
12	30.1	26.3
13	26.2	31.6
14	21.9	31.3
15	23.7	32.8
16	28.6	31.8
17	29.5	31.5
18	30.4	30.1
19	32.2	30.1
20	27.3	33.4
21	29.9	32.3
22	30.9	33.0
Average	34.8	24.6

TABLE XII  
BEHAVIOR OF SINGLE BUD PIECES WHEN PLANTED WITH THE BUDS TO THE SIDE  
(Average of 10)

Bud No.	Length of shoot (cm.)	Time for emergence (days)
1	44.0	13.4
2	48.8	12.3
3	50.0	10.9
4	49.6	11.7
5	44.7	12.2
6	41.3	12.3
7	36.3	12.9
8	39.6	13.9
9	35.3	14.3
10	35.7	15.7
11	36.5	15.5
12	29.9	19.3
13	34.9	20.8
14	27.7	23.7
15	25.7	23.3
16	25.9	24.5
17	29.1	27.7
18	28.1	27.7
19	26.8	23.6
20	27.3	25.0
21	31.7	25.3
22	27.9	30.0
Average	35.6	18.8

It is clear from the data in Tables X, XI and XII that, although all buds are capable of growth, there is a difference in vigor between the top and bottom buds. The very youngest buds were weak but under ordinary practices these buds would have been considered too soft for planting. There is no significant difference in the average shoot produced if the buds are planted "up," "down," or to the "side." Although none of these differences in the average shoot length is significant, the generalization is true that, if a single-bud seed piece is so planted that the bud is "down," it will produce a shoot at least as strong as that produced by an "up" bud. This is borne out by other tests as well. In fact, if anything the "down" bud when alone on the seed piece will tend to produce a stronger shoot than the "up" bud for reasons that will be apparent later.

The time required for the shoots to emerge is, as might be expected, in direct proportion to the distance from the surface. The "up" buds come through first with the older buds requiring about twice the number of days as the younger buds. When the buds are down, about twice the time is required as for corresponding "up" buds. The "side" buds are intermediate.

With this information, we can now proceed to the study of the influence of the number of buds and their position on the resulting germination (Tables XIII-XVII). Three series were conducted dealing with these two points. All three series showed the same results and hence only one will be reported here. In this study (Series XXXI) some exceptionally fine planting material was obtained from the Makiki Station. The 10-month old H 109 cane was cut into thirds and from these Nos. I, II and III were selected. One-, two-, three-, four-, and five-bud pieces were selected from within each age group. These were planted with the bud position as indicated in the following tables. The plantings were made April 13, 1939, and harvested May 27, 1939. It should be borne clearly in mind that all the buds planted were apparently capable of growth and that the plantings were made in a well-drained soil and under very favorable soil-temperature conditions. In other words, the only two factors operating are the number of buds on the seed piece and the position of the buds relative to one another.

Representative examples of the different groups were selected and photographed. Only No. 1 pieces were used for this purpose. In examining the accompanying illustrations, it should be remembered that in order to obtain better photographs, all the seed-piece roots were removed, but the roots developing from the young shoots were left on.

TABLE XIII  
GERMINATION OF ONE-BUD PIECES

Age of seed pieces	Position of bud	Total No. of seed pieces	Per cent emergence	Average length of shoot (cm.)
I	Up	50	100.0	70.3
I	Down	50	100.0	70.2
I	Side	50	100.0	75.4
II	Up	50	96.0	46.2
II	Down	50	94.0	38.4
II	Side	50	96.0	50.8
III	Up	50	88.0	33.4
III	Down	50	90.0	33.3
III	Side	50	90.0	31.2



Fig. 1



Fig. 2



Fig. 3

Figs. 1-3. Behavior of one-bud seed pieces. Fig. 1 (top) seed piece planted with the bud up, Fig. 2 (lower left) planted with the bud down, and Fig. 3 (lower right) planted with the bud to the side. Note roots on the shoots which curved in their growth. (All seed-piece roots removed before photographing.)

The data in Table XIII show clearly that, where only one bud is present on a seed piece, its position relative to the surface of the soil plays no part in the vigor of the shoot produced. Those pieces with the bud up (Fig. 1) produce a strong, straight shoot, while those with the bud down (Fig. 2) or to the side (Fig. 3) must, of course, curve in their growth. In general those which curve in their emergence develop shoot roots at a considerably earlier stage than do those which grow straight.

TABLE XIV  
GERMINATION OF TWO-BUD PIECES

Age of seed pieces	Position of terminal bud	Number of buds used	Per cent germination	—Average length of shoot—		
				Terminal	Basal	Average
I	Up	50	66.0	77.9	20.7	49.3
I	Down	50	94.0	47.8	71.5	59.7
I	Side	50	100.0	70.0	55.9	63.0
II	Up	50	70.0	53.0	18.7	35.9
II	Down	50	88.0	31.5	47.6	39.6
II	Side	50	94.0	48.3	42.8	45.6
III	Up	50	78.0	36.1	24.4	30.3
III	Down	50	84.0	28.6	32.6	30.6
III	Side	50	86.0	37.8	36.0	36.9

As soon as there are two buds on a seed piece, Table XIV, one affects the behavior of the other. When the terminal\* bud is up (Fig. 4), the basal bud, which automatically is down, is almost completely dominated. The result is a markedly reduced germination. The terminal bud is stronger than in the one-bud pieces indicating that some food was derived from the realm of the second bud, although the remaining data amply show that this removal of food is not the dominant influence in inhibiting the development of the second bud. This inhibiting effect upon the "down" bud is uniform throughout the series, although it is proportionally less severe in the older pieces.

The terminal bud when down, resulting in the basal bud being up, is inhibited by the basal bud although not so completely as vice versa, as is indicated by the higher germination and also the more vigorous shoot.

In some cases the terminal bud, when down, is completely inhibited (Fig. 5), while in others, it is not (Fig. 6). Strong development results where the buds are on the side (Fig. 7). Again, as the age increases, the dominance of the "up" bud is less complete.

\* The "terminal" bud on each seed piece is the one on the end of the stick nearest the growing point of the cane at the time it was cut into pieces, in contrast to the "basal" bud which is at the opposite end of the seed piece and nearer the base of the cane at the time of cutting.



Fig. 4



Fig. 5



Fig. 6



Fig. 7

Figs. 4-7. Behavior of two-bud seed pieces. Fig. 4 (upper left) seed piece planted with terminal bud up showing complete suppression of the basal bud. Fig. 5 (upper right) and Fig. 6 (lower left) planted with terminal bud down showing suppression of terminal bud in Fig. 5 and the development of both buds in Fig. 6. Fig. 7 planted with buds to the side showing strong development of both buds.



Fig. 8



Fig. 9



Fig. 10



Fig. 11

Figs. 8-11. Behavior of three-bud seed pieces. Fig. 8 (upper left) planted with terminal bud up. Fig. 9 (upper right) and Fig. 10 (lower left) planted with terminal bud down showing suppression of both terminal and basal buds in Fig. 9 and only basal bud in Fig. 10. Fig. 11 (lower right) planted with buds on the side showing strong development of all buds.

When the buds are on the side, germination is consistently better, neither bud exhibits strong dominance although in all cases the terminal shoot is stronger. In view of the results shown in Tables X, XI and XII, this might be due in part, at least, to the age of the buds rather than entirely to polar inhibition.

When the seed piece carries three buds (Table XV), the same general relations hold but, in addition, the length of the seed piece is beginning to have an unfavorable influence on germination. This can best be noted by comparing the germination of two- and three-bud pieces when the buds are on the side.

In all cases (Table XV) where the terminal bud is up (Fig. 8), it dominates the second bud which is down. Also, it has a mild inhibiting effect on the third bud which is also up. Where the terminal bud is down (Fig. 9), the third bud is also down and hence the middle bud which is up dominates the third almost completely. At times the terminal bud is dominated completely, while at other times (Fig. 10) it produces a strong shoot. But in the No. II and III pieces, it is less inhibited than it was in the 2-bud series indicating that, with two down buds sharing the inhibitory effects, the terminal bud suffers less than the basal.

When the buds are on the side (Fig. 11), total germination is better in all cases although third buds on the No. II and III pieces show marked inhibition.

TABLE XV  
GERMINATION OF THREE-BUD SEED PIECES

Age of seed pieces	Position of terminal bud	Total No. of buds used	Per cent germination	Average length of shoot			
				Terminal	Middle	Basal	Average
I	Up	75	76.0	72.1	20.4	51.6	48.0
I	Down	75	60.0	31.9	80.7	15.5	42.7
I	Side	75	93.0	57.3	47.2	45.8	50.1
II	Up	75	85.0	61.9	30.1	28.6	40.2
II	Down	75	63.0	43.0	60.6	5.4	36.3
II	Side	75	85.0	62.2	44.2	15.0	43.8
III	Up	75	72.0	52.7	13.3	36.9	34.3
III	Down	75	71.0	44.8	42.3	17.6	34.5
III	Side	75	88.0	38.4	35.9	15.3	29.9

In seed pieces carrying four buds (Table XVI), the influence of the lengthening piece is still more marked; first, by lower germination percentages and, second, by a consistently weaker average shoot. The fourth bud on the piece, when down, is completely inhibited but even when it is on the side, its development is so weak that it rarely produces a shoot which establishes itself.

When the terminal bud is up (Fig. 12), the second and fourth buds are almost completely inhibited. When the second bud is up (Fig. 13), the terminal and third buds are almost completely dominated. Though some of the terminal buds emerge, they usually fail to produce a strong shoot. When the buds are on the side, most of them emerge. Sometimes the fourth bud is completely inhibited (Fig. 14) while at other times it produces a weak shoot (Fig. 15).



Fig. 12



Fig. 13



Fig. 14



Fig. 15

Figs. 12-15. Behavior of four-bud seed pieces. Fig. 12 (upper left) planted with terminal bud up showing suppression of the two down buds. Fig. 13 planted with terminal bud down showing suppression of the two down buds. Fig. 14 (lower left) and Fig. 15 (lower right) planted with buds to the side. The length of the seed piece is beginning to affect the development of the basal bud which may be completely suppressed (Fig. 14) or may develop a somewhat weakened shoot (Fig. 15).

TABLE XVI  
GERMINATION OF FOUR-BUD SEED PIECES

Age of seed pieces	Position of terminal bud	Total		Average length of shoot				
		No. of buds used	Per cent germination	Terminal	Second	Third	Basal	Average
I	Up	100	60.0	73.5	16.9	63.8	9.1	40.8
I	Down	100	72.0	30.2	70.9	14.4	37.3	38.2
I	Side	100	94.0	59.9	51.7	28.0	23.3	40.7
II	Up	100	64.0	64.1	27.7	34.3	6.2	33.1
II	Down	100	69.0	36.2	61.5	8.3	18.6	31.1
II	Side	100	74.0	52.7	43.8	22.3	6.3	31.3
III	Up	100	60.0	45.1	16.9	32.5	8.3	25.6
III	Down	100	70.0	38.5	40.9	11.7	23.0	28.5
III	Side	100	77.0	47.9	40.5	25.0	15.3	32.2

When the seed piece carries five buds, germination is further reduced, but when the buds are on the side germination as well as shoot vigor continues to be best. When the terminal bud is up (Fig. 16), the second and fourth buds rarely emerge. When the second bud is up (Fig. 17), the third and fifth buds are completely inhibited, and the first one occasionally emerges. When the buds are on the side, sometimes under most optimum conditions each one produces a strong shoot (Fig. 18), but more commonly the fourth and fifth buds and sometimes even the third are more or less completely suppressed (Fig. 19).

When six buds are on the seed piece, the trends already shown continue. Under these circumstances the germination rarely exceeds 50 per cent (Figs. 20, 21, 22, 23). This is also true where each seed piece carries seven buds (Figs. 24, 25, 26, 27).

The trends in this phase of the study are clear. The more buds on the seed piece, the lower the germination and the lower the average shoot vigor. Further, planting the pieces with the buds on the side gives results which are consistently superior to any other position. In the first place, less inhibition occurs and, in the second place, the curved shoots produced roots at a much earlier stage. When the buds are up and down, the "down" buds produce many shoot roots but, since the shoot usually fails to emerge, these roots are wasted.

TABLE XVII  
GERMINATION OF FIVE-BUD SEED PIECES  
(125 buds used in each case)

Age of seed pieces	Position of terminal bud	Per Cent germination	Average length of shoot					
			Terminal	Second	Third	Fourth	Basal	Average
I	Up	69.0	70.8	16.0	60.9	10.4	31.3	37.9
I	Down	59.0	31.0	73.0	16.3	54.0	10.2	36.9
I	Side	83.0	66.8	63.3	47.0	38.4	12.7	45.6
II	Up	63.0	66.4	19.7	47.4	3.4	5.3	28.4
II	Down	53.0	31.3	62.5	11.3	31.3	2.8	27.8
II	Side	64.0	60.5	51.0	25.7	6.7	2.6	29.3
III	Up	62.0	42.3	20.3	30.9	3.8	10.8	21.6
III	Down	54.0	35.0	54.6	5.9	26.2	2.1	24.7
III	Side	69.0	51.4	41.9	26.7	26.9	10.2	31.4



Fig. 16



Fig. 17



Fig. 18



Fig. 19

Figs. 16-19. Behavior of five-bud seed pieces. Fig. 16 (upper left) planted with terminal bud up showing complete suppression of the two down buds and a weak shoot from the basal up bud. Fig. 17 (upper right) planted with terminal bud down showing complete suppression of the three down buds. Fig. 18 (lower left) and Fig. 19 (lower right) planted with buds to the side. The influence of the length of the seed piece is becoming very marked. Fig. 18 shows all buds developing but the fourth shoot is weak and the basal shoot very weak. Fig. 19 shows two strong shoots out of five buds.



Fig. 20



Fig. 21



Fig. 22



Fig. 23

Figs. 20-23. Behavior of six-bud seed pieces. Fig. 20 (upper left) planted with terminal bud up showing only two strong shoots. Fig. 21 (upper right) and Fig. 22 (lower left) planted with terminal bud down showing only two strong shoots in Fig. 21 and three in Fig. 22. Fig. 23 (lower right) planted with buds to the side showing three shoots of which one is weak. It is clear in this set that at least one half of the buds are wasted when six-bud pieces are used.



Fig. 24



Fig. 25



Fig. 26



Fig. 27

Figs. 24-27. Behavior of seven-bud seed pieces. Fig. 24 (upper left) planted with terminal bud up showing only two strong shoots. Fig. 25 (upper right) planted with terminal bud down showing complete suppression of five buds. Fig. 26 (lower left) planted with terminal bud up showing three shoots. Fig. 27 (lower right) planted with the buds to the side showing development of four shoots. In this set more than half of the buds are wasted.

In order to summarize more clearly the influences of length of seed piece and number of buds, the behavior of No. I seed pieces is summarized in Table XVIII. The shoots are classified as being strong (above 50.0 cm.), intermediate (between 20-50 cm.) and weak (below 20 cm.).

TABLE XVIII  
SUMMARY OF REACTION OF NO. I PIECES TO SEED-PIECE LENGTH AND  
BUD POSITION

Number of buds— Per piece	Used	Position of terminal bud	Per cent of shoots		
			Above 50 cm.	20-50 cm.	Below 20 cm.
1	50	Up	100.0	0.0	0.0
1	50	Down	94.0	4.0	2.0
1	50	Side	98.0	0.0	2.0
2	50	Up	56.0	8.0	36.0
2	50	Down	74.0	20.0	6.0
2	50	Side	76.0	24.0	0.0
3	75	Up	54.6	24.6	20.8
3	75	Down	38.6	18.8	42.6
3	75	Side	66.7	21.3	12.0
4	100	Up	49.0	7.0	44.0
4	100	Down	40.0	21.0	39.0
4	100	Side	50.0	22.0	28.0
5	125	Up	42.4	14.0	44.0
5	125	Down	37.9	16.5	45.6
5	125	Side	49.6	24.8	25.6

Since it is impractical to plant one-bud or even two-bud seed pieces, it is clear that, by using three-bud pieces and planting with the buds to the side, 66 per cent of the shoots will be strong and 20 per cent will be intermediate. As the piece is lengthened, strong shoots decrease in number and are replaced by weak shoots. Planting short pieces with the buds to the side is clearly superior to any other method.

The reader should again be reminded that all the experiments dealing with seed-piece length and bud position were carefully carried out under very favorable temperature and soil conditions. Therefore, the results obtained are to be considered as maximum germinations in each case. As the temperature drops, however, the influence of seed-piece length becomes much more marked. In some series conducted in the cold, no matter how many buds were on the seed piece, only one grew. This was always either the terminal bud or the most terminal "up" bud. All the "down" buds as well as the basal "up" buds failed to develop.

#### *Effect of the Sheaths on Germination:*

The presence of the leaf sheath (trash) on the planting piece affects germination to a slight extent. Several series of plantings were undertaken to test this point, only one of which will be reported. Since the trash would be a factor with young planting material only, top seed was used. One hundred single-bud pieces were used for each of the following series: A—the sheaths were removed, and

B—the sheaths were left attached. The planting was made June 27, 1938, and all buds were in the "up" position. Emergence records were compiled. The results are shown in Table XIX.

TABLE XIX  
INFLUENCE OF LEAF SHEATH ON GERMINATION

Condition	Germination per cent	Average time for emergence (days)
A—Without sheaths .....	100.0	10.13
B—With sheaths .....	92.0	13.18

The leaf sheath affects germination in two ways. First, some buds may remain dormant even after twenty-eight days. By this time, the seed pieces were rotting. Second, all buds under the sheath require more time to emerge. In fact, practically all the buds without sheaths had emerged before the others had started.

Whether this mild effect of the sheath is worth considering in planting techniques depends upon several factors. The cost in labor of removing the sheaths is one factor. Where other conditions for germination are good and where good planting material is available, the somewhat reduced germination and extended time for emergence are not offset by the additional work involved in removing the sheaths. But where planting conditions are unfavorable, i. e., low soil temperature, poor aeration, etc., the extra time required for emergence may mean the difference between successful and unsuccessful stands.

Another factor which must be considered is the nature of the bud. If the bud is soft and protruding, the removal of the sheath is almost certain to result in mechanical damage to the buds due to handling. Where conditions are such that the sheaths should be removed to obtain good stands, they should be removed as late in the planting routine as possible to avoid as much as possible the injury to the buds. With some varieties and under some methods of handling, the factor of mechanical injury plays a most important role in germination.

When plantings are made for experimental purposes where uniform shoots are desirable, the leaf sheaths should be removed. An analysis of the shoots resulting from the series shown in Table XIX emphasizes this point. The shoots were measured 28 days after planting, and these measurements are classified in Table XX.

TABLE XX  
EFFECT OF SHEATHS ON VIGOR AND UNIFORMITY OF SHOOTS

Planting treatment	Per cent of shoots						
	Below 5 cm.	5-15 cm.	15-25 cm.	25-35 cm.	35-45 cm.	45-55 cm.	55-65 cm.
Without sheaths .....	0	0	0	4.0	16.0	48.0	32.0
With sheaths .....	8.0	0.0	4.0	20.0	36.0	32.0	0.0

The differences here clearly indicate the uniformity of stand which obtains when sheaths are removed, and also the greater vigor of the shoots resulting. The difference between the A and B treatments is greater than can be accounted for purely on the basis of the three-day difference in emergence. This table

also emphasizes the desirability of removing the trash under conditions which are unfavorable.

Thus, whether or not to remove the trash depends entirely upon local conditions. Where rapid emergence and strong uniform stand are essential, the removal of the sheaths is important. Where conditions in the soil are marginal, the removal of the sheaths may hasten the processes sufficiently to make for a satisfactory stand. But where soil conditions are good and where the planting material has strong buds, it is doubtful whether the extra labor involved in removing the sheaths can be justified.

#### COMPOSITION OF THE PLANTING MATERIAL

Although no studies were undertaken to determine the effect of the composition of the seed pieces on their subsequent germination, observations indicate that some work should be done on this point. It is clear that, if the seed pieces are soft, therefore high in moisture and perhaps nitrogen, the buds are very susceptible to mechanical injury. Furthermore, the top seed pieces are higher in phosphorus and potassium than are the body pieces. The body pieces in turn have a greater carbohydrate content, but the top pieces have a sufficient supply of this material. Attempts to stimulate germination indicate that nitrogen is usually insufficient for best germination results.

#### STIMULATION TREATMENTS

Since planting is done about once in 10 years, it seems that it should be important and desirable to get an excellent stand from the very first. Although there are many who say that it doesn't matter whether the stand is uniform or not because the cane will fill in later with the secondary shoots, field observations by the author fail to substantiate this contention. Shoots that emerge late from the soil struggle along for perhaps two or three months and are finally shaded out. Secondary shoots which develop while the primary shoot is still very small will succeed in establishing themselves. But while one hears reports of the large number of secondary and tertiary shoots which develop in the plant crop, actual cane-by-cane examination fails to reveal any factual basis for this general belief. In connection with field work on another project, many hundreds of canes have been dug up in the field and, so far, there has not been a single tertiary shoot found which survived the early competition. Furthermore, where the initial stand was uniform, between 70-80 per cent of the final cane is made up of primary shoots and only 20-30 per cent of secondaries. These secondaries are the ones which developed very early while the primary shoot was still in the three-leaf stage. In other words, it seems clear that there is every incentive to devote enough care and attention to the planting operation to assure this good stand.

Attention has already been called to the desirability of selecting good planting time as well as material. But, under plantation conditions, it is often necessary to plant under conditions which are less than favorable. Is there anything which can be done in treating seed pieces before planting which will aid in obtaining rapid emergence of strong shoots? Considerable attention was given this problem of preplanting treatments. Although many different stimulants were tried, only the best ones will be reported. Treating seed pieces in warm water, in warm solu-

tions of calcium nitrate, ammo-phos, etc., dipping them in hot water ( $52^{\circ}$  C.) for 20 minutes, and combinations of these were used and the results are reported in the tables which follow. One series was planted in the cold, October 12, 1939. Nos. I, II and III seed pieces were selected, treated, and planted in flats with buds to the side. The daily temperature maxima averaged  $71^{\circ}$  F. This represents very unfavorable temperature conditions. In examining the data, it should be borne in mind that the planting material was cut in the morning and was in treatment before noon. Furthermore, the soaking treatments were carried out at temperatures between  $85$ – $95^{\circ}$  F. These results are tabulated in Table XXI. Twenty-five three-bud seed pieces were used in each treatment.

TABLE XXI  
INFLUENCE OF VARIOUS TREATMENTS ON GERMINATION UNDER  
UNFAVORABLE TEMPERATURE CONDITIONS ( $71.0^{\circ}$  F.)

Age of seed pieces	Treatment prior to planting	Per cent emergence	Average shoot length (cm.)	Emergence (days)
I	Control . . . . .	36.0	13.4	36.8
I	Warm water ( $85$ – $95^{\circ}$ F.)—24 hours . . .	65.3	33.2	22.8
I	Hot-water dip ( $52^{\circ}$ C.)—20 min. . . . .	49.3	21.4	29.6
I	Warm 1% $\text{Ca}(\text{NO}_3)_2$ sol. ( $85$ – $95^{\circ}$ F.)— 24 hours . . . . .	78.7	36.2	24.2
II	Control . . . . .	26.6	6.8	40.1
II	Warm water ( $85$ – $95^{\circ}$ F.)—24 hours . . .	38.7	12.5	26.0
II	Hot-water dip ( $52^{\circ}$ C.)—20 min. . . . .	38.7	11.7	34.7
II	Warm 1% $\text{Ca}(\text{NO}_3)_2$ sol. ( $85$ – $95^{\circ}$ F.)— 24 hours . . . . .	42.7	15.0	34.0
III	Control . . . . .	17.3	4.5	38.4
III	Warm water ( $85$ – $95^{\circ}$ F.)—24 hours . . .	28.0	9.4	30.8
III	Hot-water dip ( $52^{\circ}$ C.)—20 min. . . . .	29.3	8.9	36.2
III	Warm 1% calcium nitrate sol. ( $85$ – $95^{\circ}$ F.)— —24 hours . . . . .	44.0	6.4	37.0

There are several points of interest shown in this table. In the first place, treating the seed pieces prior to planting under unfavorable temperature conditions results in a material stimulation both in germination and in shoot growth. Furthermore, the influence of age on response to stimulation is marked. Body pieces, although they respond to treatment so far as emergence is concerned, show little response in shoot vigor. It seems clear from this that, where plantings have to be made under unfavorable conditions, nothing but the best top seed pieces should be used.

Soaking in warm water, dipping in hot water, or soaking in a warm solution of calcium nitrate are all very helpful although the last is the most effective.

To demonstrate the effect of treating the seed pieces in warm solutions, another series was undertaken in which cold water was used for the water treatment and the calcium nitrate solution. The hot-water dip was the same as used in the last table, and then one lot of 153 buds was dipped in hot water for 20 minutes and put to soak for 24 hours in a cold ( $65$ – $75^{\circ}$  F.) solution of calcium nitrate. The results are reported in Table XXII. One hundred and fifty-three buds were used

in each treatment. Three-bud seed pieces were selected from No. I planting material. The soil temperature maxima average 69.8° F.

TABLE XXII

EFFECTS OF COLD PREPLANTING TREATMENTS ON SUBSEQUENT GERMINATION  
UNDER UNFAVORABLE SOIL TEMPERATURE (69.8° F.)

Treatment	Per cent emergence
Control .....	9.8
Cold water (65–75° F.)—24 hours.....	17.0
Cold 1% calcium nitrate solution (65–75° F.)—24 hours.....	32.0
Hot-water dip (52° C.)—20 minutes.....	60.8
Hot-water dip + 24 hours in cold 1% calcium nitrate (65–75° F.).....	69.9

The importance of heat in the treatment is clearly demonstrated. Furthermore, it is clear that plantings under such very adverse temperature conditions without prior treatment are doomed to complete replanting.

Still another low-temperature series was set up June 20, 1939, and harvested August 13, 1939. In this series, in addition to the soaking treatments, one series was planted with the buds to the side and another with the terminal bud up in half the cases and down in the other half. The results are reported in Table XXIII.

TABLE XXIII

INFLUENCE OF VARIOUS TREATMENTS ON GERMINATION UNDER  
UNFAVORABLE TEMPERATURE CONDITIONS

Age of seed pieces	Treatment	Length of shoot average (cm.)		Per cent emergence	
		Buds side	Buds up and down	Buds side	Buds up and down
I	Control .....	25.9	21.0	58.6	44.6
I	Warm water (85–95° F.)—24 hours.....	30.6	28.9	65.3	46.1
I	Warm 1% Ca (NO <sub>3</sub> ) <sub>2</sub> (85–95° F.)—24 hours..	35.9	36.1	74.6	50.0
I	Warm 1% KH <sub>2</sub> PO <sub>4</sub> (85–95° F.)—24 hours....	38.7	26.8	69.3	56.7

This series again shows the desirability of treating seed pieces when planting under unfavorable conditions. The soaking with a warm (85–95° F.) solution of calcium nitrate is clearly superior to the others. Furthermore, the response to treatment under unfavorable growing conditions is more marked when the seed pieces are planted with the buds to the side.

It appears to be clear, then, that where plantings must be made under unfavorable soil temperatures, untreated seed pieces will give very unsatisfactory stands whereas any one of the following treatments will give comparatively satisfactory results: soaking in warm water (85–95° F.) for 24 hours; hot-water dip, 52° C., for 20 minutes, and soaking in a warm calcium nitrate solution (1%) for 24 hours. Even though all three treatments are helpful, the warm calcium nitrate solution is uniformly superior to the others. As a result of these stimulation treatments, it is clear that only top seed pieces should be used, and that it is desirable to plant the seed pieces with the buds to the side.

One further point remains to be examined. Is it worth while to treat seed pieces when planting under very favorable conditions? This point was subjected to several series of experiments only three of which will be reported here.

Series XXVI was planted under very nearly perfect conditions of soil temperature and texture. The planting was made October 5, 1938, and harvested November 13, 1938. The results of this test are shown in Table XXIV.

TABLE XXIV  
EFFECT OF PREPLANTING TREATMENTS ON GERMINATION UNDER  
FAVORABLE CONDITIONS (91.8° F.)

Age of seed pieces	Treatment	Per cent emergence	Avg. shoot length (cm.)	Average emergence (days)
I	Control .....	93.3	49.0	10.3
	Warm water (85-95° F.)—24 hours	96.7	55.1	8.8
	Warm water (85-95° F.)—48 hours	94.5	58.7	8.6
	Warm 1% sol. (85-95° F.)—			
	Ammo-Phos—24 hours .....	99.3	58.9	8.6
	Warm 1% sol. (85-95° F.)— calcium nitrate—24 hours.....	99.3	65.3	7.7
II	Control .....	74.4	30.0	13.5
	Warm water (85-95° F.)—24 hours	82.7	44.2	10.2
	Warm water (85-95° F.)—48 hours	88.0	46.1	9.3
	Warm 1% sol. (85-95° F.)—			
	Ammo-Phos—24 hours .....	84.0	46.3	9.2
	Warm 1% sol. (85-95° F.)— calcium nitrate—24 hours.....	89.3	48.4	10.1
III	Control .....	77.8	39.3	13.8
	Warm water (85-95° F.)—24 hours	74.7	37.2	11.7
	Warm water (85-95° F.)—48 hours	84.4	39.8	10.5
	Warm 1% sol. (85-95° F.)—			
	Ammo-Phos—24 hours .....	88.0	52.2	12.1
	Warm 1% sol. (85-95° F.)— calcium nitrate—24 hours.....	93.3	45.2	11.0

In this series, the controls were so nearly perfect that opportunities for improvement were correspondingly reduced. But it is apparent that, at least for experimental purposes where rapid germination and a uniformly strong stand is desired, it may be worth treating the seed pieces prior to planting.

Series XXXVIII, planted June 15, 1939, and harvested August 3, is another study of the same type. The temperature was somewhat lower than that for Series XXVI, and the soil heavier. The results are reported in Table XXV.

TABLE XXV  
EFFECT OF PREPLANTING TREATMENTS ON GERMINATION UNDER  
FAVORABLE CONDITIONS (88° F.)

Age of seed pieces	Treatment	Per cent emergence	Average shoot length
I	Control .....	85.3	62.9
	Warm water (85-95° F.)—24 hours.....	89.3	63.1
	Warm water (85-95° F.)—48 hours.....	96.0	64.3
	Warm 1% $\text{KH}_2\text{PO}_4$ (85-95° F.)—24 hours.....	90.6	53.8
	Warm 1% $\text{Ca}(\text{NO}_3)_2$ (85-95° F.)—24 hours.....	100.0	68.2
III	Control .....	65.3	48.5
	Warm water (85-95° F.)—24 hours.....	66.0	51.9
	Warm water (85-95° F.)—48 hours.....	77.3	54.6
	Warm 1% $\text{KH}_2\text{PO}_4$ (85-95° F.)—24 hours.....	70.7	57.1
	Warm 1% $\text{Ca}(\text{NO}_3)_2$ (85-95° F.)—24 hours.....	72.0	55.3

Here again some stimulation obtains—but probably the preplanting treatment is not worth while since a completely satisfactory stand may be obtained without prior treatment.

Series XLIV was planted October 12, 1939, and harvested December 9, 1939. Since the phosphate treatment is less satisfactory than the other treatments, it was dropped and the hot-water dip was substituted. The results are shown in Table XXVI. Cold water and solutions were used. Following ten days of favorable temperatures, a rainy period developed and the average temperature maxima fell from 90–95° F. to 75–80° F.

TABLE XXVI  
EFFECT OF COLD PREPLANTING TREATMENTS ON SUBSEQUENT  
GERMINATION UNDER FAVORABLE CONDITIONS

Age of seed pieces	Treatment	Per cent emergence	Average shoot length	Emergence (days)
I	Control .....	88.0	58.2	15.6
	Cold water (65–75° F.)—24 hours.....	86.7	54.5	13.8
	Hot-water dip (52° C.)—20 minutes....	89.3	52.2	17.0
	Cold 1% calcium nitrate sol. (65–75° F.) —24 hours .....	93.3	60.8	12.4
II	Control .....	73.3	41.3	17.8
	Cold water (65–75° F.)—24 hours.....	76.0	41.3	14.7
	Hot-water dip (52° C.)—20 minutes....	72.0	38.3	22.7
	Cold 1% calcium nitrate sol. (65–75° F.) —24 hours .....	78.7	47.1	17.0
III	Control .....	54.7	33.5	20.4
	Cold water (65–75° F.)—24 hours.....	53.3	36.6	20.5
	Hot-water dip (52° C.)—20 minutes....	66.7	40.3	19.9
	Cold 1% calcium nitrate sol. (65–75° F.) —24 hours .....	62.7	31.3	18.1

It is clearly shown here that preplanting treatments involving cold treatments are not at all satisfactory. No benefits result and, in fact, they may even retard germination.

#### GENERAL DISCUSSION

To obtain satisfactory stands of sugar cane under Hawaiian conditions requires a program developed as a result of an analysis of conditions obtaining in each field. It seems clear that given good planting material the external conditions of soil temperature, moisture, and aeration are the most important factors affecting germination results. Where soils are warm and well drained, though holding adequate moisture, successful stands may easily be obtained without any particular precautions or treatments.

To assume, however, that soil temperatures are generally favorable in Hawaii is to overlook the single largest obstacle to successful germination. During the winter months and other cloudy periods when the sun rarely strikes the soil directly, the temperatures of the soil are marginal and even submarginal for bud development. It would appear desirable, therefore, to know the soil temperatures

before planting. If temperatures are unfavorable, several practices are available to the grower who must plant under such conditions. Shallow planting of top seed pieces, which have been treated previously, will assure a reasonably good stand where complete failure may follow lackadaisical methods.

In soils which are heavy and which show a tendency to puddle, planting must be carefully done. If such soils are irrigated, aeration and soil moisture may be easily controlled by withholding water. But on unirrigated fields, heavy soils present a difficult problem. In such soils, temperature and aeration may both be limiting factors which can only be circumvented by planting previously treated seed pieces very near the surface of the soil or even by burying the butts of the pieces and allowing the tips to protrude into the air. In any event, success will depend upon the proper recognition of the limiting factors. Depth of planting together with previously treated young planting material seem to be the controlling factors in such a state of circumstances. To plant haphazardly under such conditions is a waste of effort and planting material for the replanting percentage will be high.

Several factors affecting germination which are internal to the seed piece are of importance. The age of the material is of considerable influence. Top pieces are, of course, the best. Under otherwise favorable conditions, body pieces will yield satisfactory results. But again, under unfavorable conditions, only the best material should be used for two reasons: In the first place, body pieces yield very unsatisfactory stands and they do not respond satisfactorily to treatment. Where it is necessary to use body material, it can only be emphasized that it should be segregated from the top material and planted very heavily.

The length of the seed pieces, i. e., the number of buds per seed piece, as well as their position on the planted stick impose certain effects on the subsequent growth which should be taken into consideration in planting. The results given above clearly indicate that, the longer the seed piece, the lower the germination percentage and the lower the average shoot vigor. To use pieces with more than three buds is to waste the extra buds. Furthermore, to plant seed pieces with buds in any position is also to waste nearly all of the buds in the "down" position.

The presence of trash on the seed piece slows up emergence and also reduces germination. Whether it is worth while removing this trash will depend entirely on local conditions. One word of caution should be expressed here. In some varieties, there is a tendency for nodes produced in late summer and fall to be without buds. Where trash is not removed, it may be possible to plant material without buds.

The composition of the planting material is a factor whose importance can only be surmised at this time. Material which is too soft and succulent damages easily and also rots easily. On the other hand, hard material is slow in germinating. It would seem desirable that cane to be used for planting should be grown specifically for that purpose. Such cane could be grown for a period of about six months, then hardened off for about a month before using. This would assure active buds and at the same time buds which would not be so subject to mechanical damage. Perhaps after a month of the hardening treatment, a liberal application

of nitrogen could be made. After ten days or two weeks, the material would be ready for cutting and planting.

Several treatments are available for treating seed pieces before planting, particularly in unfavorable conditions. Mere soaking in warm water (85-95° F.) for 24 or 48 hours gives substantially improved germination and vigor, but soaking in a warm one per cent solution of calcium nitrate clearly assures one of an improved stand and vigor. The "hot-water dip" (20 min. at 52° C.) results in stimulation but appears to be somewhat less effective than the calcium nitrate treatment.

In conclusion, it may be stated that germination is a physiological process which has certain minimum requirements. Unless these requirements are met, germination will be unsatisfactory. Where soil conditions are all very favorable, the idiosyncrasies of the buds may be regarded with impunity, but where they are less than favorable, these minimum requirements must be respected. At once, one hears the objection that planters cannot afford to plant carefully; that to plant at a certain depth, short pieces, with buds to the side, will add too much expense to the process. In answer to this, two things should be pointed out. In the first place, replanting is also expensive. There is the cost of the second planting itself, and then also the resulting uneven stand. In the second place, where machines are used, there seems no reason why a machine cannot be devised which will lay the pieces in the soil in precisely the right position and the correct depth. With such a machine, careful planting should be only a little more expensive than at present and perhaps actually less expensive if replanting can be eliminated.

Division of Plant Physiology  
University of Hawaii.

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## Sugar Prices

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96° CENTRIFUGALS FOR THE PERIOD  
DECEMBER 16, 1939 TO MARCH 15, 1940

Date	Per pound	Per ton	Remarks
Dec. 16, 1939.....	2.95¢	\$59.00	Puerto Ricos.
" 27.....	2.85	57.00	Cubas, 2.84, 2.86.
" 28.....	2.83	56.60	Cubas.
" 29.....	2.81	56.20	Cubas.
Jan. 3, 1940.....	2.82	56.40	Philippines.
" 4.....	2.835	56.70	Philippines, 2.83; Cubas, 2.84.
" 5.....	2.82	56.40	Philippines.
" 6.....	2.82	56.40	Cubas, 2.84, 2.80.
" 8.....	2.80	56.00	Cubas.
" 9.....	2.805	56.10	Puerto Ricos, 2.80; Cubas, 2.81.
" 10.....	2.81	56.20	Cubas.
" 12.....	2.86	57.20	Cubas.
" 15.....	2.8825	57.65	Philippines, 2.85, 2.87; Puerto Ricos, 2.90; Cubas, 2.91.
" 16.....	2.885	57.70	Philippines, 2.87; Cubas, 2.90.
" 18.....	2.905	58.10	Philippines, 2.91; Puerto Ricos, 2.90.
" 20.....	2.90	58.00	Philippines.
" 23.....	2.86	57.20	Cubas, 2.87; Puerto Ricos, 2.85.
" 24.....	2.85	57.00	Puerto Ricos.
" 30.....	2.855	57.10	Philippines, 2.85; Cubas, 2.86.
" 31.....	2.85	57.00	Philippines.
Feb. 1.....	2.815	56.30	Puerto Ricos, 2.83; Cubas, 2.80.
" 5.....	2.81	56.20	Philippines.
" 6.....	2.80	56.00	Puerto Ricos.
" 9.....	2.82	56.40	Philippines.
" 10.....	2.825	56.50	Cubas, 2.83; Philippines, 2.82; Puerto Ricos.
" 13.....	2.82	56.40	Philippines.
" 16.....	2.83	56.60	Cubas.
" 17.....	2.83	56.60	Philippines.
" 21.....	2.855	57.10	Cubas, 2.86; Puerto Ricos, 2.85; Philippines.
" 23.....	2.85	57.00	Puerto Ricos.
" 26.....	2.88	57.60	Cubas, 2.89; Puerto Ricos, 2.87.
" 28.....	2.86	57.20	Cubas.
Mar. 1.....	2.85	57.00	Puerto Ricos.
" 4.....	2.855	57.10	Puerto Ricos, 2.85; Cubas, 2.86.
" 6.....	2.845	56.90	Puerto Ricos, 2.85; Cubas, 2.84.
" 8.....	2.805	56.10	Puerto Ricos, 2.81, 2.80.
" 13.....	2.81	56.20	Philippines; Puerto Ricos.
" 15.....	2.815	56.30	Puerto Ricos, 2.81; Cubas, 2.82.

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